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01000085.9	28 March 2001 (28.03.2001)	EP	(71) Applicant (for all designated States except US): DSM N.V.		
01000087.5	28 March 2001 (28.03.2001)	EP	[NL/NL]; Het Overloon 1, NL-6411 TE Heerlen (NL).		
01000088.3	28 March 2001 (28.03.2001)	EP			
01000156.8	21 May 2001 (21.05.2001)	EP	(72) Inventors; and		
01000159.2	21 May 2001 (21.05.2001)	EP	(75) Inventors/Applicants (for US only): EDENS, Luppó		
01000160.0	21 May 2001 (21.05.2001)	EP	[NL/NL]; Hoflaan 118, NL-3062 JL Rotterdam (NL).		
01000162.6	21 May 2001 (21.05.2001)	EP	DIJK VAN, Albertus, Alard [NL/NL]; Cederdreef 32,		
01000165.9	21 May 2001 (21.05.2001)	EP	NL-3137 PB Vlaardingen (NL). KRUBASIK, Philipp		
01000166.7	21 May 2001 (21.05.2001)	EP	[DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE).		
01000168.3	21 May 2001 (21.05.2001)	EP	ALBERMANN, Kaj [DE/DE]; Lochhamer Str. 11, 82152		
01000225.1	20 June 2001 (20.06.2001)	EP	Martinsried (DE). STOCK, Alex [DE/DE]; Lochhamer		
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01000237.6	21 June 2001 (21.06.2001)	EP	KLUGBAUER, Sabine [DE/DE]; Lochhamer Str. 11,		
01000238.4	21 June 2001 (21.06.2001)	EP	82152 Martinsried (DE). WAGNER, Christian [DE/DE];		
01000240.0	21 June 2001 (21.06.2001)	EP	Lochhamer Str. 11, 82152 Martinsried (DE). FRITZ,		
01000242.6	21 June 2001 (21.06.2001)	EP	Andreas [DE/DE]; Lochhamer Str. 11, 82152 Martinsried		
01000244.2	21 June 2001 (21.06.2001)	EP	(DE). GUSTEDT VON, Wilk [DE/DE]; Lochhamer		
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(54) Title: NOVEL GENES ENCODING NOVEL PROTEOLYTIC ENZYMES

(57) Abstract: The invention relates to newly identified gene sequences that encode novel proteases obtainable from *Aspergillus niger*. The invention features the full length gene sequence of the novel genes, their cDNA sequences as well as the full-length functional protein and fragments thereof. The invention also relates to methods of using these enzymes in industrial processes and methods of diagnosing fungal infections. Also included in the invention are cells transformed with DNA according to the invention and cells wherein a protease according to the invention is genetically modified to enhance or reduce its activity and/or level of expression.

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[DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). **HOPPER, Sylvia** [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). **KEMMNER, Wolfram** [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). **TAN, Pamela** [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). **STIEBLER, Josephine** [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). **ALBANG, Richard** [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE).

(74) Agent: **HABETS, Winand, Johannes, Antonius**; DSM Patents & Trademarks, P.O. Box 9, NL-6160 MA Geleen (NL).

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NOVEL GENES ENCODING NOVEL PROTEOLYTIC ENZYMES

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Field of the invention

The invention relates to newly identified polynucleotide sequences comprising genes that encode novel proteases isolated from *Aspergillus niger*. The invention features the full length nucleotide sequence of the novel genes, the cDNA sequences comprising the full length coding sequences of the novel proteases as well as the amino acid sequences of the full-length functional proteins and fragments and variants thereof. The invention also relates to methods of using these enzymes in industrial processes and methods of diagnosing fungal infections. Also included in the invention are cells transformed with a polynucleotide according to the invention and cells wherein a protease according to the invention is genetically modified to enhance or reduce its activity and/or level of expression.

Background of the invention

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Proteolytic Enzymes

Proteins can be regarded hetero-polymers that consist of amino acid building blocks connected by a peptide bond. The repetitive unit in proteins is the central alpha carbon atom with an amino group and a carboxyl group. Except for glycine, a so-called amino acid side chain substitutes one of the two remaining alpha carbon hydrogen atoms. The amino acid side chain renders the central alpha carbon asymmetric. In general, in proteins the L-enantiomer of the amino acid is found. The following terms describe the various types of polymerized amino acids. *Peptides* are short chains of amino acid residues with defined sequence. Although there is not really a maximum to the number of residues, the term usually indicates a chain which properties are mainly determined by its amino acid composition and which does not have a fixed three-dimensional conformation. The term *polypeptide* is usually used for the longer chains, usually of defined sequence and length and in principle of the appropriate length to fold into a three-dimensional structure. *Protein* is reserved for polypeptides that occur naturally and exhibit a defined three-dimensional structure. In case the proteins main function is to catalyze a chemical reaction it usually is called an *enzyme*. Proteases are the

enzymes that catalyze the hydrolysis of the peptide bond in (poly)peptides and proteins.

Under physiological conditions proteases catalyse the hydrolysis of the peptide bond.

- 5 The International Union of Biochemistry and Molecular Biology (1984) has recommended to use the term *peptidase* for the subset of peptide bond hydrolases (Subclass E.C 3.4.). The terms *protease* and *peptide hydrolase* are synonymous with *peptidase* and may also be used here. *Proteases* comprise two classes of enzymes: the endo-peptidases and the exo-peptidases, which cleave peptide bonds at points
- 10 within the protein and remove amino acids sequentially from either N or C-terminus respectively. *Proteinase* is used as a synonym for *endo-peptidase*. The peptide bond may occur in the context of di-, tri-, tetra-peptides, peptides, polypeptides or proteins. In general the amino acid composition of natural peptides and polypeptides comprises 20 different amino acids, which exhibit the L-configuration (except for glycine which does
- 15 not have a chiral centre). However the proteolytic activity of proteases is not limited to peptides that contain only the 20 natural amino acids. Peptide bonds between so-called non-natural amino acids can be cleaved too, as well as peptide bonds between modified amino acids or amino acid analogues. Some proteases do accept D enantiomers of amino acids at certain positions. In general the remarkable stereo-
- 20 selectivity of proteases makes them very useful in the process of chemical resolution. Many proteases exhibit interesting side activities such as esterase activity, thiol esterase activity and (de)amidase activity. These side activities are usually not limited to amino acids only and might turn out to be very useful in bioconversions in the area of fine chemicals.

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- There are a number of reasons why proteases of filamentous fungi, eukaryotic microorganisms, are of particular interest. The basic process of hydrolytic cleavage of peptide bonds in proteins appears costly and potentially detrimental to an organism if not properly controlled. The desired limits to proteolytic action are achieved through the
- 30 specificity of proteinases, by compartmentalization of proteases and substrates within the cell, through modification of the substrates allowing recognition by the respective proteases, by regulation via zymogen activation, and the presence or absence of specific inhibitors, as well through the regulation of protease gene expression. In fungi, proteases are also involved in other fundamental cellular processes, including intracellular protein
- 35 turnover, processing, translocation, sporulation, germination and differentiation. In fact, *Aspergillus nidulans* and *Neurospora crassa* have been used as model organisms for

analyzing the molecular basis of a range of physiological and developmental processes. Their genetics enable direct access to biochemical and genetical studies, under defined nutrient and cultivation conditions. Furthermore, a large group of fungi pathogenic to humans, live-stock and crop, has been isolated and proteolysis has been suggested to play a role in their pathogenicity (host penetration, countering host defense mechanisms and/or nutrition during infection). Proteases are also frequently used in laboratory, clinical and industrial processes; both microbial and non-microbial proteases are widely used in the food industry (baking, brewing, cheese manufacturing, meat tenderizing), in tanning industry and in the manufacture of biological detergents (Aunstrup, 1980). The commercial interest in exploiting certain filamentous fungi, especially the *Aspergilli*, as hosts for the production of both homologous and heterologous proteins, has also recently renewed interests in fungal proteases (van Brunt, 1986ab). Proteases often cause problems in heterologous expression and homologous overexpression of proteins in fungi. In particular, heterologous expression is hampered by the proteolytic degradation of the expressed products by homologous proteases. These commercial interests have resulted in detailed studies of proteolytic spectra and construction of protease deficient strains and have improved the knowledge about protease expression and regulation in these organisms. Consequently there is a great need to identify and eliminate novel proteases in filamentous fungi.

Micro-organisms such as for example fungi are particularly useful in the large scale production of proteins. In particular when such proteins are secreted into the medium. Proteolytic enzymes play a role in these production processes. On the one hand particular proteolytic enzymes are in general required for proper processing of the target protein and the metabolic well-being of the production host. On the other hand proteolytic degradation may significantly decrease the yield of secreted proteins. Poor folding in the secretion pathway may lead to degradation by intracellular proteases. This might be a particular problem with producing heterologous proteins. The details of the proteolytic processes, which are responsible for the degradation of the proteins that are diverted from the secretory process in fungi are not exactly known. In eukaryotes the degradation of cellular proteins is achieved by a proteasome and usually involves ubiquitin labelling of proteins to be degraded. In fungi, proteasomal and vacuolar proteases are also likely candidates for the proteolytic degradation of poorly folded secretory proteins. The proteolytic degradation is likely cytoplasmic, but endoplasmic reticulum resident proteases cannot be excluded. From the aspect of production host strain improvement the proteolytic system may be an interesting target for genetic

engineering and production strain improvement. Additional copies of protease genes, over-expression of certain proteases, modification of transcriptional control, as well as knock out procedures for deletion of protease genes may provide a more detailed insight in the function a given protease. Deletion of protease encoding genes can be a valuable strategy for host strain improvement in order to improve production yield for homologous as well as heterologous proteins.

Eukaryotic microbial proteases have been reviewed by North (1982). More recently, Suarez Rendueles and Wolf (1988) have reviewed the *S. cerevisiae* proteases and their function.

Apart from the hydrolytic cleavage of bonds, proteases may also be applied in the formation of bonds. Bonds in this aspect comprise not only peptide and amide bonds but also ester bonds. Whether a protease catalyses the cleavage or the formation of a particular bond does in the first place depend on the thermodynamics of the reaction. An enzyme such as a protease does not affect the equilibrium of the reaction. The equilibrium is dependent on the particular conditions under which the reaction occurs. Under physiological conditions the thermodynamics of the reactions is in favour of the hydrolysis of the peptide due to the thermodynamically very stable structure of the zwitterionic product. By application of physical-chemical principles to influence the equilibrium, or by manipulating the concentrations or the nature of the reactants and products, or by exploiting the kinetic parameters of the enzyme reaction it is possible to apply proteases for the purpose of synthesis of peptide bonds. The addition of water miscible organic solvents decreases the extent of ionisation of the carboxyl component, thereby increasing the concentration of substrate available for the reaction. Biphasic systems, water mimetics, reverse micelles, anhydrous media, or modified amino and carboxyl groups to invoke precipitation of products are often employed to improve yields. When the proteases with the right properties are available the application of proteases for synthesis offers substantial advantages. As proteases are stereo-selective as well as regio-selective, sensitive groups on the reactants do usually not need protection and reactants do not need to be optically pure. As conditions of enzymatic synthesis are mild, racemization and decomposition of labile reactants or products can be prevented. Apart from bonds between amino acids, also other compounds exhibiting a primary amino group, a thiol group or a carboxyl group may be linked by properly selected proteases. In addition esters, thiol esters and amides may be synthesized by certain proteases. Protease have been shown to exhibit

regioselectively in the acylation of mono, di- and tri- saccharides, nucleosides, and riboflavin. Problems with stability under the sometimes harsh reaction conditions may be prevented by proper formulation. Encapsulation and immobilisation do not only stabilise enzymes but also allow easy recovery and separation from the reaction
5 medium. Extensive crosslinking, treatment with aldehydes or covering the surface with certain polymers such as dextrans, polyethyleneglycol, polyimines may substantially extend the lifetime of the biocatalyst.

The Natural Roles of Proteases

10 Traditionally, proteases have been regarded as degrading enzymes, capable of cleaving proteins into small peptides and/or amino acids, and whose role it is to digest nutrient protein or to participate in the turnover of cellular proteins. In addition, it has been shown that proteases also play key roles in a wide range of cellular processes, via mechanisms of selective modification by limited proteolysis, and thus can have essential regulatory
15 functions (Holzer and Tschensche 1979; Holzer and Heinrich, 1980). The specificity of a proteinase is assumed to be closely related to its physiological function and its mode of expression. With respect to the function of a particular protease, its localisation is often very important; for example, a lot of the vacuolar and periplasmic proteases are involved in protein degradation, while many of the membrane-bound proteases are important in
20 protein processing (Suarez Rendueles and Wolf, 1988). The different roles of proteases in many cellular processes can be divided into four main functions of proteases: 1) protein degradation, 2) posttranslational processing and (in)activation of specific proteins, 3) morphogenesis, and 4) pathogenesis.

25 An obvious role for proteases in organisms which utilise protein as a nutrient source is in the hydrolysis of nutrients. In fungi, this would involve the degradation outside the cells by extracellular broad specificity proteases. Protein degradation is also important for rapid turnover of cellular proteins and allows the cell to remove abnormal proteins and to adapt their complement of protein to changing physiological conditions. Generally,
30 proteases of rather broad specificity should be extremely well-controlled in order to protect the cell from random degradation of other than correct target proteins.

Contrary to the hydrolysis the synthesis of polypeptides occurs *in vivo* by an ATP driven process on the ribosome. Ultimately the sequence in which the amino acids are
35 linked is dictated by the information derived from the genome. This process is known as the transcription. Primary translation products are often longer than the final functional

products, and after the transcription usually further processing of such precursor proteins by proteases is required. Proteases play a key role in the maturation of such precursor proteins to obtain the final functional protein. In contrast to the very controlled trimming and reshaping of proteins, proteases can also be very destructive and may completely degrade polypeptides into peptides and amino acids. In order to avoid that proteolytic activity is unleashed before it is required, proteases are subject to extensive regulation. Many proteases are synthesized as larger precursors known as zymogens, which become activated when required. Remarkably this activation always occurs by proteolysis. Apart from direct involvement in the processing, selective activation and inactivation of individual proteins are well-known phenomena catalyzed by specific proteases.

The selectivity of limited proteolysis appears to reside more directly in the proteinase-substrate interaction. Specificity may be derived from the proteolytic enzyme which recognizes only specific amino acid target sequences. On the other hand, it may also be the result of selective exposure of the 'processing site' under certain conditions such as pH, ionic strength or secondary modifications, thus allowing an otherwise non-specific protease to catalyze a highly specific event. The activation of vacuolar zymogens by limited proteolysis gives an example of the latter kind.

Morphogenesis or differentiation can be defined as a regulated series of events leading to changes from one state to another in an organism. Although direct relationships between proteases and morphological effects could not be established in many cases, the present evidence suggests a significant involvement of proteases in fungal morphogenesis; apart from the observed extensive protein turnover during differentiation, sporulation and spore germination, proteases are thought to be directly involved in normal processes as hyphal tip branching and septum formation, (Deshpande, 1992).

Species of *Aspergillus*, in particular *A. fumigatus* and *A. flavus*, have been implicated as the causative agents of a number of diseases in humans and animals called aspergillosis (Bodey and Vartivarian, 1989). It has been repeatedly suggested that proteases are involved in virulence of *A. fumigatus* and *A. flavus* like there are many studies linking secreted proteases and virulence of bacteria. In fact, most human infections due to *Aspergillus* species are characterised by an extensive degradation of the parenchyma of the lung which is mainly composed of collagen and elastin (Campbell et al., 1994). Research has been focussed on the putative role of the secreted proteases in virulence

of *A. fumigatus* and *A. flavus* which are the main human pathogens and are known to possess elastinolytic and collagenic activities (Kolattukudy et al., 1993). These elastinolytic activities were shown to correlate *in vitro* with infectivity in mice (Kothary et al., 1984). Two secreted proteases are known to be produced by *A. fumigatus* and *A. flavus*, an alkaline serine protease (ALP) and a neutral metallo protease (MEP). In *A. fumigatus* both the genes encoding these proteases were isolated, characterised and disrupted (Reicherdt et al., 1990; Tang et al., 1992, 1993; Jatón-Ogay et al., 1994). However, *alp mep* double mutants showed no differences in pathogenicity when compared with wild type strains. Therefore, it must be concluded that the secreted *A. fumigatus* proteases identified *in vitro* are not essential factors for the invasion of tissue (Jatón Ogay et al., 1994). Although *A. fumigatus* accounts for only a small proportion of the airborne mould spores, it is the most frequently isolated fungus from lung and sputum (Schmitt et al., 1991). Other explanations for the virulence of the fungus could be that the conditions in the bronchia (temperature and nutrients) are favourable for the parasitic growth of *A. fumigatus*. As a consequence, invasive aspergillosis could be a circumstantial event, when the host pathogenic defences have been weakened by immunosuppressive treatments or diseases like AIDS.

Four major classes of proteases are known and are designated by the principal functional groups in their active site: the 'serine', the 'thiol' or 'cysteine', the 'aspartic' or 'carboxyl' and the 'metallo' proteases. A detailed state of the art review on these major classes of proteases, minor classes and unclassified proteases can be found in Methods in Enzymology part 244 and 248 (A.J.Barrett ed, 1994 and 1995).

Specificity of Proteases

Apart from the catalytic machinery of proteases another important aspect of proteolytic enzymes is the specificity of proteases. The specificity of a protease indicates which substrates the protease is likely to hydrolyze. The twenty natural amino acids offer a large number of possibilities to make up peptides. Eg with twenty amino acids one can make up already 400 dipeptides and 800 different tripeptide, and so on. With longer peptides the number of possibilities will become almost unlimited. Certain proteases hydrolyze only particular sequences at a very specific position. The interaction of the protease with the peptide substrate may encompass one up to ten amino acid residues of the peptide substrate. With large proteinacious substrates there may be even more residues of the substrate that interact with the proteases. However this likely involves less specific interactions with protease residues outside the active site binding cleft. In

general the specific recognition is restricted to the linear peptide, which is bound in the active site of the protease.

The nomenclature to describe the interaction of a substrate with a protease has been introduced in 1967 by Schechter and Berger (Biochem. Biophys. Res. Com., 1967, 27, 157-162) and is now widely used in the literature. In this system, it is considered that the amino acid residues of the polypeptide substrate bind to so-called *sub-sites* in the active site. By convention, these sub-sites on the protease are called S (for sub-sites) and the corresponding amino acid residues are called P (for peptide). The amino acid residues of the N-terminal side of the scissile bond are numbered P3, P2, P1 and those residues of the C-terminal side are numbered P1', P2', P3'. The P1 or P1' residues are the amino acid residues located near the scissile bond. The substrate residues around the cleavage site can then be numbered up to P8. The corresponding sub-sites on the protease that complement the substrate binding residues are numbered S3, S2, S1, S1', S2', S3', etc, etc. The preferences of the sub-sites in the peptide binding site determine the preference of the protease for cleaving certain specific amino acid sequences at a particular spot. The amino acid sequence of the substrate should conform with the preferences exhibited by the sub-sites. The specificity towards a certain substrate is clearly dependant both on the binding affinity for the substrate and on the velocity at which subsequently the scissile bond is hydrolysed. Therefore the specificity of a protease for a certain substrate is usually indicated by its k_{cat}/K_m ratio, better known as the specificity constant. In this specificity constant k_{cat} represents the turn-over rate and K_m is the dissociation constant.

Apart from amino acid residues involved in catalysis and binding, proteases contain many other essential amino acid residues. Some residues are critical in folding, some residues maintain the overall three dimensional architecture of the protease, some residues may be involved in regulation of the proteolytic activity and some residue may target the protease for a particular location. Many proteases contain outside the active site one or more binding sites for metal ions. These metal ions often play a role in stabilizing the structure. In addition secreted eukaryotic microbial proteases may be extensively glycosylated. Both N- and O-linked glycosylation occurs. Glycosylation may aid protein folding, may increase solubility, prevent aggregation and as such stabilize the mature protein. In addition the extent of glycosylation may influence secretion as well as water binding by the protein.

Regulation of Proteolytic Activity

A substantial number of proteases are subject to extensive regulation of the proteolytic activity in order to avoid undesired proteolytic damage. To a certain extent this regulation takes place at transcription level. For example in fungi the transcription of secreted protease genes appears to be sensitive to external carbon and nitrogen sources, whereas genes encoding intracellular proteases are insensitive. The extracellular pH is sensed by fungi and some genes are regulated by pH. In this process transcriptional regulator proteins play a crucial role. Proteolytic processing of such regulator proteins is often the switch that turns the regulator proteins either on or off.

Proteases are subject to intra- as well as intermolecular regulation. This implies certain amino acids in the proteolytic enzyme molecule that are essential for such regulation. Proteases are typically synthesized as larger precursors known as zymogens, which are catalytically inactive. Usually the peptide chain extension rendering the precursor protease inactive is located at the amino terminus of the protease. The precursor is better known as pro-protein. As many of the proteases processed in this way are secreted from the cells they contain in addition a signal sequence (pre sequence) so that the complete precursor is synthesized as a pre-pro-protein. Apart from rendering the protease inactive the pro-peptide often is essential for mediating productive folding. Examples of proteases include serine proteases (alpha lytic protease, subtilisin, aqualysin, prohormone convertase), thiol proteases (cathepsin L and cruzian), aspartic proteases (proteinase A and cathepsin D) and metalloproteases. In addition the pro-peptide might play a role in cellular transport either alone or in conjunction with signal peptides. It may facilitate interaction with cellular chaperones or it may facilitate transport over the membrane. The size of the extension in the precursor pre-pro-protein may vary substantially, ranging from a short peptide fragment to a polypeptide, which can exist as an autonomous folding unit. In particular these larger extensions are often observed to be strong inhibitors of the protease even after cleavage from the protease. It was observed that even after cleavage such pro-peptides could assist in proper folding of the proteases. As such pro-peptides can be considered to function as molecular chaperones and separate or additional co-expression of such pro-peptides could be advantageous for protease production.

There is substantial difference in the level of regulation between proteases that are secreted into the medium and proteases that remain intracellular. Proteases secreted

into the medium are usually after activation no longer subject to control and therefore are usually relatively simple in their molecular architecture consisting of one globular module. Intracellular proteases are necessarily subject to continuous control in order to avoid damage to the cells. In contrast with zymogens of secreted proteases in more

5 complex regulatory proteases very large polypeptide segments may be inserted between the signal and the zymogen activation domain of the proteolytic module. Structure-function studies indicate that such non-protease parts may be involved in interactions with macroscopic structures, membranes, cofactors, substrates, effectors, inhibitors, ions, that regulate activity and activation of the proteolytic module(s) or its

10 (their) zymogens. The non-proteolytic modules exhibit remarkable variation in size and structure. Many of the modules can exist as such independently from the proteolytic module. Therefore such modules can be considered to correspond to independent structural and functional units that are autonomous with respect to folding. The value of such a modular organization is that acquisition of new modules can endow the recipient

15 protease with new novel binding specificities and can lead to dramatic changes in its activity, regulation and targeting. The principle of modular organized proteolytic enzymes may also be exploited by applying molecular biology tools in order to create novel interactions, regulation, specificity, and/or targeting by shuffling of modules. Although in general such additional modules are observed as N or C terminal

20 extension, also large insertions within the exterior loops of the catalytic domain have been observed. It is believed that also in this case the principal fold of the protease represents still the essential topology to form a functional proteolytic entity and that the insertion can be regarded as substructure folded onto the surface of the proteolytic module.

25

Molecular Structure

In principle the modular organization of larger proteins is a general theme in nature. In particular within the larger multimodular frameworks typical proteolytic modules show sizes of 100 to 400 amino acids on the average. This corresponds with the average

30 size of most of the globular proteolytic enzymes that are secreted into the medium. As discussed above polypeptide modules are polypeptide fragments, which can fold and function as independent entities. Another term for such modules is domains. However domain is used in a broader context than module. The term domain as used herein refers usually to a part of the polypeptide chain that depicts in the three-dimensional

35 structure a typical folding topology. In a protein domains interact to varying extents, but less extensively than do the structural elements within domains. Other terms such as

subdomain and folding unit are also used in literature. As such it is observed that many proteins that share a particular functionality may share the same domains. Such domains can be recognized from the primary structure that may show certain sequence patterns, which are typical for a particular domain. Typical examples are the mononucleotide binding fold, cellulose binding domains, helix-turn-helix DNA binding motif, zinc fingers, EF hands, membrane anchors. Modules refer to those domains which are expected to be able to fold and function autonomously. A person skilled in the art knows how to identify particular domains in a primary structure by applying commonly available computersoftware to said structure and homologous sequences from other organisms or species.

Although multimodular or multidomain proteins may appear as a string of beads, assemblies of substantial more complex architecture have been observed. In case the various beads reside on the same polypeptide chain the beads are generally called modules or domains. When the beads do not reside on one and same polypeptide chain but form assemblies via non-covalent interactions then the term *subunit* is used to designate the bead. Subunits may be transcribed by one and the same gene or by different genes. The multi-modular protein may become proteolytically processed after transcription leading to multiple subunits. Individual subunits may consist of multiple domains. Typically the smaller globular proteins of 100-300 amino acids usually consist only of one domain.

Molecular Classification of Proteolytic Enzymes

In general proteases are classified according to their molecular properties or according to their functional properties. The molecular classification is based on the primary structure of the protease. The primary structure of a protein represents its amino acid sequence, which can be derived from the nucleotide sequence of the corresponding gene. Tracing extensively the similarities in the primary structures may allow for the notice of similarities in catalytic mechanism and other properties, which even may extend to functional properties. The term *family* is used to describe a group of proteases that show evolutionary relationship based on similarity between their primary structures. The members of such a family are believed to have arisen by divergent evolution from the same ancestor. Within a family further sub-grouping of the primary structures based on more detailed refinement of sequence comparisons results in

subfamilies. Classification according to three-dimensional fold of the proteases may comprise secondary structure, tertiary structure and quaternary structure. In general the classification on secondary structure is limited to content and gross orientation of secondary structure elements. Similarities in tertiary structure have led to the

5 recognition of superfamilies or clans. A superfamily or a clan is a group of families that are thought to have common ancestry as they show a common 3-dimensional fold. In general tertiary structure is more conserved than the primary structure. As a consequence similarity of the primary structure does not always reflect similar functional properties. In fact functional properties may have diverged substantially

10 resulting in interesting new properties. At present quaternary structure has not been applied to classify various proteases. This might be due to a certain bias of the structural databases towards simple globular proteases. Many proteolytic systems that are subject to activation, regulation, or complex reaction cascades are likely to consist of multiple domains or subunits. General themes in the structural organization of such

15 protease systems may lead to new types of classification.

Classification according to specificity.

In absence of sequence information proteases have been subject to various type of

20 functional classification. The classification and naming of enzymes by reference to the reactions which are catalyzed is a general principle in enzyme nomenclature. This approach is also the underlying principle of the EC numbering of enzymes (*Enzyme Nomenclature* 1992 Academic Press, Orlando). Two types of proteases (EC 3.4) can be recognized within *Enzyme Nomenclature* 1992, those of the exo-peptidases (EC

25 3.4.11-19) and those of the endo-peptidases (EC 3.4.21-24, 3.4.99). Endo-peptidases cleave peptide bonds in the inner regions of the peptide chain, away from the termini. Exo-peptidases cleave only residues from the ends of the peptide chain. The exo-peptidases acting at the free N-terminus may liberate a single amino acid residue, a dipeptide or a tripeptide and are called respectively amino peptidases (EC 3.4.11),

30 dipeptidyl peptidases (EC 3.4.14) and tripeptidyl peptidase (EC 3.4.14). Proteases starting peptide processing from the carboxyl terminus liberating a single amino acid are called carboxy peptidase (EC 3.4.16-18). Peptidyl-dipeptidases (EC 3.4.15) remove a dipeptide from the carboxyl terminus. Exo- and endo-peptidase in one are the dipeptidases (EC 3.4.13), which cleave specifically only dipeptides in their two amino

35 acid halves. Omega peptidases (EC 3.4.19) remove terminal residues that are either substituted, cyclic, or linked by isopeptide bonds

Apart from the position where the protease cleaves a peptide chain, for each type of protease a further division is possible based on the nature of the preferred amino acid residues in the substrate. In general one can distinguish proteases with broad, medium and narrow specificity. Some proteases are simply named after the specific proteins or polypeptides that they hydrolyze, e.g. keratinase, collagenase, elastase. A narrow specificity may pin down to one particular amino acid or one particular sequence which is removed or which is cleaved respectively. When the protease shows a particular preference for one amino acid in the P1 or P1' position the name of this amino acid may be a qualifier. For example prolyl amino peptidase removes proline from the amino terminus of a peptide (proline is the P1 residue). X-Pro or proline is used when the bond on the imino side of the proline is cleaved (proline is P1' residue), eg proline carboxypeptidase removes proline from the carboxyl terminus. Prolyl endopeptidase (or Pro-X) cleaves behind proline while proline endopeptidase (X-Pro) cleaves in front of a proline. Amino acid residue *in front of* the scissile peptide bond refers to the amino acid residue that contributes the carboxyl group to the peptide bond. The amino acids residue *behind* the scissile peptide bond refers to the amino acid residue that contributes the amino group to the peptide bond. According to the general convention an amino acid chain runs from amino terminus (the start) to the carboxyl terminus (the end) and is numbered accordingly. Endo proteases may also show clear preference for a particular amino acid in the P1 or P1' position, eg glycyl endopeptidase, peptidyl-lysine endopeptidase, glutamyl endopeptidase. In addition proteases may show a preference for a certain group of amino acids that share a certain resemblance. Such a group of preferred amino acids may comprise the hydrophobic amino acids, only the bulky hydrophobic amino acids, small hydrophobic, or just small amino acids, large positively charged amino acids, etc, etc. Apart from preferences for P1 and P1' residues also particular preferences or exclusions may exist for residues preferred by other subsites on the protease. Such multiple preferences can result in proteases that are very specific for only those sequences that satisfy multiple binding requirements at the same time. In general it should be realized that protease are rather promiscuous enzymes. Even very specific protease may cleave peptides that do not comply with the generally observed preference of the protease. In addition it should be realized that environmental conditions such as pH, temperature, ionic strength, water activity, presence of solvents, presence of competing substrates or inhibitors may influence the preferences of the proteases. Environmental condition may not only influence the protease but also influence the way the proteinaceous substrate is presented to the protease.

Classification by catalytic mechanism.

- Proteases can be subdivided on the basis of their catalytic mechanism. It should be understood that for each catalytic mechanism the above classification based on specificity leads to further subdivision for each type of mechanism. Four major classes of proteases are known and are designated by the principal functional group in the active site: the serine proteases (EC 3.4.21 endo peptidase, EC 3.4.16 carboxy peptidase), the thiol or cysteine proteases (EC 3.4.22 endo peptidase, EC 3.4.18 carboxy peptidase), the carboxyl or aspartic proteases (EC 3.4.23 endo peptidase) and metallo proteases (EC 3.4.24 endo peptidase, EC 3.4.18 carboxy peptidase). There are characteristic inhibitors of the members of each catalytic type of protease. These small inhibitors irreversibly modify an amino acid residue of the protease active site. For example, the serine protease are inactivated by Phenyl Methane Sulfonyl Fluoride (PMSF) and Diisopropyl Fluoro Phosphate (DFP), which react with the active Serine whereas the chloromethylketone derivatives react with the Histidine of the catalytic triad. Phosphoramidon and 1,10 Phenanthroline typically inhibit metallo proteases. Inhibition by Pepstatin generally indicates an aspartic protease. E64 inhibits thiol protease specifically. Amastatin and Bestatin inhibit various aminopeptidases.
- Substantial variations in susceptibility of the proteases to the inhibitors are observed, even within one catalytic class. To a certain extent this might be related to the specificity of the protease. In case binding site architecture prevents a mechanism based inhibitor to approach the catalytic site, then such a protease escapes from inhibition and identification of the type of mechanism based on inhibition is prohibited.
- Chymostatin for example is a potent inhibitor for serine protease with chymotrypsin like specificity, Elastatinal inhibits elastase like serine proteases and does not react with trypsin or chymotrypsin, 4 amido PMSF (APMSF) inhibits only serine proteases with trypsin like specificity. Extensive accounts of the use of inhibitors in the classification of proteases include Barret and Salvesen, *Proteinase Inhibitors*, Elsevier Amstardam, 1986; Bond and Beynon (eds), *Proteolytic Enzymes, A Practical Approach*, IRL Press, Oxford, 1989; Methods in Enzymology, eds E.J.Barret, volume 244, 1994 and volume 248, 1995; E.Shaw, *Cysteinylnl proteinases and their selective inactivation*, Adv Enzymol. 63:271-347 (1990)
- Classification according to optimal performance conditions.

The catalytic mechanism of a proteases and the requirement for its conformational integrity determine mainly the conditions under which the protease can be utilized. Finding the protease that performs optimal under application conditions is a major challenge. Often conditions at which proteases have to perform are not optimal and do
5 represent a compromise between the ideal conditions for a particular application and the conditions which would suit the protease best. Apart from the particular properties of the protease it should be realized that also the presentation of a proteinaceous substrates is dependant on the conditions, and as such determines also which conditions are most effective for proteolysis. Specifications for the enzyme that are
10 relevant for application comprise for example the pH dependence, the temperature dependence, sensitivity for or the dependence of metal ions, ionic strength, salt concentration, solvent compatibility. Another factor of major importance is the specific activity of a protease. The higher the enzyme's specific activity, the less enzyme is needed for a specific conversion. Lower enzyme requirements imply lower costs and
15 lower protein contamination levels.

The pH is a major parameter that determines protease performance in an application. Therefor pH dependence is an important parameter to group proteases. The major groups that are recognized are the acid proteases, the neutral proteases, the alkaline proteases and the high alkaline proteases. The optimum pH matches only to some
20 extent the proteolytic mechanism, eg aspartic protease show often an optimum at acidic pH, metalloproteases and thiol proteases often perform optimal around neutral pH to slightly alkaline, serine peptidases are mainly active in the alkaline and high alkaline region. For each class exceptions are known. In addition the overall water activity of the system plays a role. The pH optimum of a protease is defined as the pH
25 range where the protease exhibits an optimal hydrolysis rate for the majority of its substrates in a particular environment under particular conditions. This range can be narrow, e.g. one pH unit, as well as quite broad, 3-4 pH units. In general the pH optimum is also dependant on the nature of the proteinaceous substrate. Both the turnover rate as well as the specificity may vary as a function of pH. For a certain
30 efficacy it can be desirable to use the protease far from its pH optimum because production of less desired peptides is avoided. Less desired peptides might be for example very short peptides or peptides causing a bitter taste. In addition a more narrow specificity can be a reason to choose conditions that deviate from optimal conditions with respect to turnover rate. Dependant on the pH the specificity may be
35 narrow, e.g. only cleaving the peptide chain in one particular position or before or after one particular amino acid, or broader, e.g. cleaving a chain at multiple positions or

cleaving before or after more different types of amino acids. In fact the pH dependence might be an important tool to regulate the proteolytic activity in an application. In case the pH shifts during the process the proteolysis might cease spontaneously without the need for further treatment to inactivate the protease. In some cases the proteolysis
5 itself may be the driver of the pH shift.

Very crucial for application of proteases is their handling and operating stability. As protease stability is strongly affected by the working temperature, stability is often also referred to as thermostability. In general the stability of a protease indicates how long a
10 protease retains its proteolytic activity under particular conditions. Particular conditions may comprise fermentation conditions, conditions during isolation and down stream processing of the enzyme, storage conditions, formulation and operating or application conditions. In case particular conditions encompass elevated temperatures stability in general refers to thermostability. Apart from the general causes for enzyme inactivation
15 such as chemical modification, unfolding, aggregation etc, main problem with proteases is that they are easy subject to autodegradation. Especially for the utilization of proteases the temperature optimum is a relevant criterion to group proteases. Although there are different definitions, economically the most useful definition is the temperature or the temperature range in which the protease is most productive in a
20 certain application. Protease productivity is a function of both the stability and the turnover rate. Where elevated temperature in general will increase the turnover rate, rapid inactivation will counteract the increase in turnover rate and ultimately lead to low productivity. The conformational stability of the protease under a given process condition will determine its maximum operating temperature. The temperature at which
25 the protease loses its active conformation, often indicated as unfolding or melting point, can be determined according various methods, for example NMR, Circular Dichroism Spectroscopy, Differential Scanning Calorimetry etc etc. For protease unfolding is usually accompanied by a tremendous increase in autodegradation rate.

30 In applications where low temperatures are required protease may be selected with emphasis on a high intrinsic activity at low to moderate temperature. As under such conditions inactivation is relatively slow, under these conditions activity might largely determine productivity. In processes where only during a short period protease activity is required, the stability of the protease might be used as a switch to turn the protease
35 off. In such case more labile instead of very thermostable protease might be preferred.

Other environmental parameters which may play a role in selecting the appropriate protease may be its sensitivity to salts. The compatibility with metal ions which are found frequently at low concentrations in various natural materials can be crucial for certain applications. In particular with metallo proteases certain ions may replace the catalytic metal ion and reduce or even abolish activity completely. In some applications metal ions have to be added on purpose in order to prevent the washout of the metal ions coordinated to the protease. It is well known that for the sake of enzyme stability and life-time, calcium ions have to be supplied in order to prevent dissociation of protein bound calcium.

10

Most microorganisms show a certain tolerance with respect to adapting to changes in the environmental condition. As a consequence at least the proteolytic spectrum that the organism is able to produce are likely to show at least similar tolerances. Such a proteolytic spectrum might be covered by many proteases covering together the whole proteolytic spectrum or by only a few proteases of a broad spectrum. Taking into account the whole proteolytic spectrum of a microorganism it can be very important to take the location into account.

15

Cellular localisation and characterization of proteolytic processing and degradation

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From an industrial point of view the proteases which are excreted from the cell have specific advantages with respect to producibility at a large scale and stress tolerance as they have to survive without protection of the cell. The large group of cellular protease can be further subdivided in soluble and membrane bound. Membrane bound may comprise protease at the inside as well the outside of the membrane. Intracellular soluble protease may be subdivided further according to specific compartments of the cell where they do occur. As the cell shields the proteases to some extent from the environment and because the cell controls the conditions in the cell, intracellular protease might be more sensitive to large environmental changes and their optima might correlate better with the specific intracellular conditions. Knowing the conditions of the cellular department where the protease resides might indicate their preferences. Where extracellular protease in general do not require any regulation any more once excreted from the cell, intracellular proteases are often subject to more complicated control and regulation.

25

30

With respect to the function of a particular protease, its localisation is often very

important; for example, a lot of the vacuolar and periplasmic proteases are involved in protein degradation, while many of the membrane-bound proteases are important in protein processing (Suarez Rendueles and Wolf, 1988).

- 5 A comprehensive review on the biological properties and evolution of proteases has been published in van den Hombergh: Thesis Landbouwwuniversiteit Wageningen: An analysis of the proteolytic system in *Aspergillus* in order to improve protein production ISBN 90-5485-545-2, which is hereby incorporated by reference herein.

10 The protease problem

An important reason for the interest in microbial proteases are protease related expression problems observed in several expression hosts used in bioprocess industry. The increasing use of heterologous hosts for the production of proteins, by recombinant
15 DNA technology, has recently brought this problem into focus, since it seems that heterologous proteins are more prone to proteolysis (Archer et al., 1992; van den Hombergh et al., 1996b).

In *S. cerevisiae*, already in the early eighties the protease problem and the involvement
20 of several proteases, thus complicating targetted gene disruption approaches to overcome this problem, was recognised. During secretion a protein is exposed to several proteolytic activities residing in the secretory pathway. Additionally, in a prototrophic microorganism as *Aspergillus* secreted proteins can be exposed to several extracellular proteolytic activities

25

The problem of degradation of heterologously expressed proteins is well documented in *Aspergillus* (van den Hombergh Thesis Landbouwwuniversiteit Wageningen: An analysis of the proteolytic system in *Aspergillus* in order to improve protein production ISBN 90-5485-545-2) and has been reported in the expression of cow prochymosin,
30 human interferon α -2 tPA, GMCSF, IL6, lactoferrin, chicken egg-white lysosyme, porcine pIA2, *A. niger* pectin lyase B, *E. coli* enterotoxin B and β -glucuronidase, and *Erwinia carotovora* pectate lyase 3.

The problem of proteolysis may be addressed at several stages in protein production.
35 Bioprocess engineers may address the problem of proteolysis by downstream

processing at low temperatures , by early separation of product and protease(s) or by use of protease inhibitors. These may all lead to successful reduction of the problem. However it is certainly not eliminated, because much of the degradation occurs *in vivo* during the production of the protein.

5

In understanding how proteolysis is controlled in the cell, a major question concerns the recognition mechanism by which proteolysis is triggered. Into what extent are proteolytically susceptible (heterologous) proteins recognised as aberrant because of misfolding or, if correctly folded, as 'foreign', because they do not possess features essential for stability which are specific to the host. Various types of stress can cause the overall proteolysis in a cell to increase significantly. Factors known to increase rate of proteolysis include nutrient starvation and various other types of stress (i.e. elevation of temperature, osmotic stress, toxic substances and expression of certain heterologous proteins). To deal with proteolysis-related expression problems *in vivo*, several approaches have been proven successful as will be discussed below. However, we have to keep in mind that true 'non-proteolytic cells' cannot exist, since proteolysis by intracellular proteases is involved in many essential metabolic and 'housekeeping' reactions. Reducing proteolysis will therefore always be a process in which the changed genetical background which results in decreased proteolytic has to be analysed for potential secondary effects which could lead to reduced protein production (e.g. reduced growth rate or sporulation).

Disruption of proteases in filamentous fungal expression hosts

Berka and coworkers (1990) describe the cloning and disruption of the *A. awamori pepA* gene. More recently, three disrupted aspartyl proteases in *A. niger* have been described. Disruptants for both the major extracellular aspartyl proteases and the major vacuolar aspartyl protease were described. Double and triple disruptants were generated via recombination and tested for protease spectra and expression and secretion of the *A. niger* pectin lyase PELB protein, which is very susceptible to proteolytic degradation (van den Hombergh et al., 1995). Disruption of *pepA* and *pepB* resulted both in reduction of extracellular protease activities, 80% and 6 %, respectively. In the $\Delta pepE$ disruptant also other (vacuolar) protease activities were severely affected caused by inactivating of the proteolytic cascade for other vacuolar proteases. Reduced extracellular activities correlated with reduced *in vitro* degradation of PELB and improved *in vivo* expression of *pepB* (van den Hombergh et al., 1996f).

Protease deficient (prt) mutants filamentous fungi

Several *Aspergillus* protease deficient mutants have been studied whether protein production is improved. Archer and coworkers describe the reduced proteolysis of Hen egg white lysozyme in supernatants of an *A. niger* double *prt* mutant generated by
5 Mattern and coworkers (1992) and conclude that although the degradation is not absent, it is significantly reduced. Van den Hombergh et al. (1995) show that the *in vitro* degradation of *A. niger* PELB is reduced in all seven *prt* complementation groups they have isolated. Virtually no degradation is observed in the *prtB*, *prtF* and *prtG* mutants. Recently, the expression of the *pebB* gene was shown to be improved in six
10 complementation groups tested (*prtA-F*) and highest expression levels were observed in the *prtB*, *prtF* and *prtG* mutants. In addition to the single mutants, which contained residual extracellular proteolytic activities varying from 2-80 % compared to wild type activity, double mutants were generated both by recombination and by additional rounds of mutagenesis. Via this approach several double *prt* mutants were selected and further
15 characterised, which showed a further reduction of PELB degradation compared to their parental strains.

Instead of elimination of protease activities via disruption or mutagenesis, reduced proteolysis can also be achieved via down-regulation of the interfering proteolytic
20 activities. This may be achieved by genetically altering the promoter or other regulatory sequences of the gene. As shown by Fraissinet-Tachet and coworkers (1996) the extracellular proteases in *A. niger* are all regulated by carbon catabolite repression and nitrogen metabolite repression. Nutrient starvation also causes the overall proteolysis rate in a cell to increase strongly, which makes sense for a cell that lacks nutrients but
25 possesses proteins, that under starvation conditions are not needed or needed only in smaller amounts. In expression strategies which allow high expression on media containing high glucose and ammonium concentrations reduced proteolysis has been reported. Several constitutive glycolytic promoters (*gpd* and *pkiA*) are highly expressed under these conditions and can also be used to drive (heterologous) gene expression in
30 continuous fermentations. The type of nutrient starvation imposed can influence different proteases to varying extent, which means that the importance of nutrient conditions in a given process depend on the type of proteolysis that is involved. Specific proteolysis may therefore be induced by conditions of substrate limitation which are frequently used in many large-scale fermentation processes.

35

The protease problem can nowadays be addressed in part by one or more of the above

strategies. However, the residual proteolytic activity of yet unidentified proteolytic enzymes still constitutes a major problem in the art. In order to further reduce the level of unwanted proteolysis, there is a great need in the art to identify novel proteases responsible for degradation of homologously and heterologously expressed proteins.

5 This invention provides such novel protease gene sequences encoding novel proteases. Once the primary sequence of a novel protease gene is known, one or more of the above recombinant DNA strategies may be employed to produce (knock-out) mutants with reduced proteolytic activity.

10 Despite the widespread applications of proteases in a great number of industrial processes, current enzymes also have significant shortcomings with respect to at least one of the following properties.

When added to animal feed, current proteases are not sufficiently resistant to digestive
15 enzymes present in the gastrointestinal (GI) tract of e.g. pigs and poultry.

With respect to another aspect, the currently available enzymes are not sufficiently resistant to specific (high) temperatures and (high) pressure conditions that are applied during extrusion or pelleting operations.

20

Also, the current enzymes are not sufficiently active in a pH range of 3-7, conditions prevailing in many food, beverage products as well as in in the GI tract of most animals.

25 According to yet another aspect the specificity of the currently available proteases is very limited which results in the inability of the existing enzymes to degrade or to dissolve certain "protease resistant" proteins thus resulting in low peptide or amino acid yields. Moreover proteases with new specificities allow the synthesis of new peptides.

30 Yet another drawback of the currently available enzymes is their low specific activity.

It is therefore clear that for a large number of applications a strong desire exists for proteases that are more resistant to digestive enzymes, high temperature and/or pressure and which exhibit novel specificities regarding their sites of hydrolysis. The
35 present invention provides such enzymes.

Object of the invention

It is an object of the invention to provide novel polynucleotides encoding novel proteases. A further object is to provide naturally and recombinantly produced
5 proteases as well as recombinant strains producing these. Such strains may also be used to produce classical fermentation products faster or with higher yields. Yet another object of the invention is to provide a filamentous fungus strain defective in producing a protease according to the invention. Such strains may be used for a more efficient production of heterologous or homologous proteins. Also antibodies and fusion
10 polypeptides are part of the invention as well as methods of making and using the polynucleotides and polypeptides according to the invention.

Summary of the invention

15 The invention provides for novel polynucleotides encoding novel proteases.

More in particular, the invention provides for polynucleotides having a nucleotide sequence that hybridises (preferably under highly stringent conditions) to a sequence according to a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ
20 ID NO: 57 or to a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114. Consequently, the invention provides nucleic acids that are about 60%, preferably 65%, more preferably 70%, even more preferably 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% homologous to the sequences according to a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a
25 sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114.

In a more preferred embodiment the invention provides for such an isolated polynucleotide obtainable from a filamentous fungus, preferably *Aspergilli*, in particular *A. niger* is preferred.

30 In one embodiment, the invention provides for an isolated polynucleotide comprising a nucleic acid sequence encoding a polypeptide with an amino acid sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 or functional equivalents thereof.

35 In a further preferred embodiment, the invention provides an isolated polynucleotide

encoding at least one functional domain of a polypeptide according to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 or functional equivalents thereof.

- 5 In a preferred embodiment the invention provides a protease gene according to a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57. In another aspect the invention provides a polynucleotide, preferably a cDNA encoding an *A. niger* protease selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 or variants or fragments of that polypeptide. In a preferred embodiment the
- 10 cDNA has a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 or functional equivalents thereof.

- A genomic clone encoding a polypeptide according to the invention may also be obtained by selecting suitable probes to specifically amplify a genomic region
- 15 corresponding to any of the sequences according to SEQ ID NO: 1 to SEQ ID NO: 57 or fragments thereof, hybridising that probe under suitable conditions to genomic DNA obtained from a suitable organism, such as *Aspergillus*, e.g. *A. niger*, amplifying the desired fragment e.g. by PCR (polymerase chain reaction) followed by purifying and cloning of the amplified fragment.

20

In an even further preferred embodiment, the invention provides for a polynucleotide comprising the coding sequence of the genomic polynucleotides according to the invention, preferred is a polynucleotide sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114.

25

In another preferred embodiment, the invention provides a cDNA obtainable by cloning and expressing a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 into a suitable host organism, such as *A. niger*.

- 30 A polypeptide according to the invention may also be obtained by cloning and expressing a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 into a suitable host organism, such as *A. niger*.

- The invention also relates to vectors comprising a polynucleotide sequence according
- 35 to the invention and primers, probes and fragments that may be used to amplify or detect the DNA according to the invention.

In a further preferred embodiment, a vector is provided wherein the polynucleotide sequence according to the invention is functionally linked with regulatory sequences suitable for expression of the encoded amino acid sequence in a suitable host cell, such as *A. niger* or *A. oryzae*. The invention also provides methods for preparing polynucleotides and vectors according to the invention.

The invention also relates to recombinantly produced host cells that contain heterologous or homologous polynucleotides according to the invention.

In one embodiment, the invention provides recombinant host cells wherein the expression of a protease according to the invention is significantly reduced or wherein the activity of the protease is reduced or wherein the protease is even inactivated. Such recombinants are especially useful for the expression of homologous or heterologous proteins.

In another embodiment, the invention provides recombinant host cells wherein the expression of a protease according to the invention is significantly increased or wherein the activity of the protease is increased. Such recombinants are especially useful for the expression of homologous or heterologous proteins where maturation is seriously hampered in case the required proteolytic cleavage becomes the rate limiting step.

In another embodiment the invention provides for a recombinantly produced host cell that contains heterologous or homologous DNA according to the invention, preferably DNA encoding proteins bearing signal sequences and wherein the cell is capable of producing a functional protease according to the invention, preferably a cell capable of over-expressing the protease according to the invention, for example an *Aspergillus* strain comprising an increased copy number of a gene or cDNA according to the invention.

In another embodiment the invention provides for a recombinantly produced host cell that contains heterologous or homologous DNA according to the invention and wherein the cell is capable of secreting a functional protease according to the invention, preferably a cell capable of over-expressing and secreting the protease according to the invention, for example an *Aspergillus* strain comprising an increased copy number of a gene or cDNA according to the invention.

In yet another aspect of the invention, a purified polypeptide is provided. The polypeptides according to the invention include the polypeptides encoded by the polynucleotides according to the invention. Especially preferred is a polypeptide
5 according to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 or functional equivalents thereof.

The invention also provides for antibodies reactive with a polypeptide according to the invention. These antibodies may be polyclonal, yet especially preferred are monoclonal
10 antibodies. Such antibodies are particularly useful for purifying the polypeptides according to the invention.

Fusion proteins comprising a polypeptide according to the invention are also within the scope of the invention. The invention also provides methods of making the
15 polypeptides according to the invention.

The invention further relates to a method for diagnosing aspergillosis either by detecting the presence of a polypeptide according to the invention or functional equivalents thereof, or by detecting the presence of a DNA according to the invention
20 or fragments or functional equivalents thereof.

The invention also relates to the use of the protease according to the invention in an industrial process as described herein

25

Detailed description of the invention

Polynucleotides

30 The present invention provides polynucleotides encoding proteases having an amino acid sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 or functional equivalents thereof. The sequence of these genes was determined by sequencing a genomic clone obtained from *Aspergillus niger*. The invention provides polynucleotide sequences comprising the gene encoding these proteases as well as
35 their complete cDNA sequence and its coding sequence. Accordingly, the invention

relates to an isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 or functional equivalents thereof.

5

More in particular, the invention relates to an isolated polynucleotide hybridisable under stringent conditions to a polynucleotide selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 preferably under highly stringent conditions.

10

Advantageously, such polynucleotides may be obtained from filamentous fungi, in particular from *Aspergillus niger*. More specifically, the invention relates to an isolated polynucleotide having a nucleotide sequence according to a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114.

15

The invention also relates to an isolated polynucleotide encoding at least one functional domain of a polypeptide according to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 or functional equivalents thereof.

20

As used herein, the terms "gene" and "recombinant gene" refer to nucleic acid molecules which may be isolated from chromosomal DNA, which include an open reading frame encoding a protein, e.g. an *A. niger* protease. A gene may include coding sequences, non-coding sequences, introns and regulatory sequences. Moreover, a gene refers to an isolated nucleic acid molecule as defined herein.

25

A nucleic acid molecule of the present invention, such as a nucleic acid molecule having the nucleotide sequence of a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 or a functional equivalent thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. For example, using all or portion of the nucleic acid sequence of a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or the nucleotide sequence of a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 as a hybridization probe, nucleic acid molecules according to the invention can be isolated using standard hybridization and cloning techniques (e.g., as described in Sambrook, J., Fritsch, E. F., and Maniatis, T. *Molecular Cloning: A*

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Laboratory Manual.2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989).

Moreover, a nucleic acid molecule encompassing all or a portion of a sequence
5 selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence
selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 can be
isolated by the polymerase chain reaction (PCR) using synthetic oligonucleotide
primers designed based upon the sequence information contained in a sequence
selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence
10 selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114.

A nucleic acid of the invention can be amplified using cDNA, mRNA or alternatively,
genomic DNA, as a template and appropriate oligonucleotide primers according to
standard PCR amplification techniques. The nucleic acid so amplified can be cloned
15 into an appropriate vector and characterized by DNA sequence analysis.

Furthermore, oligonucleotides corresponding to or hybridisable to nucleotide
sequences according to the invention can be prepared by standard synthetic
techniques, e. g., using an automated DNA synthesizer.

20

In a preferred embodiment, an isolated nucleic acid molecule of the invention
comprises the nucleotide sequence shown in a sequence selected from the group
consisting of SEQ ID NO: 58 to SEQ ID NO: 114. The sequence of a sequence
selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 corresponds
25 to the coding region of the A. niger protease cDNA. This cDNA comprises sequences
encoding the A. niger protease polypeptide according to a sequence selected from the
group consisting of SEQ ID NO: 115 to SEQ ID NO: 171.

In another preferred embodiment, an isolated nucleic acid molecule of the invention
30 comprises a nucleic acid molecule which is a complement of the nucleotide sequence
shown in a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID
NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID
NO: 114 or a functional equivalent of these nucleotide sequences.

35 A nucleic acid molecule which is complementary to another nucleotide sequence is one
which is sufficiently complementary to the other nucleotide sequence such that it can

hybridize to the other nucleotide sequence thereby forming a stable duplex.

One aspect of the invention pertains to isolated nucleic acid molecules that encode a polypeptide of the invention or a functional equivalent thereof such as a biologically
5 active fragment or domain, as well as nucleic acid molecules sufficient for use as hybridisation probes to identify nucleic acid molecules encoding a polypeptide of the invention and fragments of such nucleic acid molecules suitable for use as PCR primers for the amplification or mutation of nucleic acid molecules.

10 An "isolated polynucleotide" or "isolated nucleic acid" is a DNA or RNA that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. Thus, in one embodiment, an isolated nucleic acid includes some or all of the 5' non-coding (e.g., promotor) sequences that are
15 immediately contiguous to the coding sequence. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (e.g., a cDNA or a genomic DNA fragment produced by PCR or restriction endonuclease treatment) independent of other
20 sequences. It also includes a recombinant DNA that is part of a hybrid gene encoding an additional polypeptide that is substantially free of cellular material, viral material, or culture medium (when produced by recombinant DNA techniques), or chemical precursors or other chemicals (when chemically synthesized). Moreover, an "isolated nucleic acid fragment" is a nucleic acid fragment that is not naturally occurring as a
25 fragment and would not be found in the natural state.

As used herein, the terms "polynucleotide" or "nucleic acid molecule" are intended to include DNA molecules (e.g., cDNA or genomic DNA) and RNA molecules (e.g., mRNA) and analogs of the DNA or RNA generated using nucleotide analogs. The
30 nucleic acid molecule can be single-stranded or double-stranded, but preferably is double-stranded DNA. The nucleic acid may be synthesized using oligonucleotide analogs or derivatives (e.g., inosine or phosphorothioate nucleotides). Such oligonucleotides can be used, for example, to prepare nucleic acids that have altered base-pairing abilities or increased resistance to nucleases.

35

Another embodiment of the invention provides an isolated nucleic acid molecule which

is antisense to a protease nucleic acid molecule, e.g., the coding strand of a protease nucleic acid molecule. Also included within the scope of the invention are the complement strands of the nucleic acid molecules described herein.

5 Sequencing errors

The sequence information as provided herein should not be so narrowly construed as to require inclusion of erroneously identified bases. The specific sequences disclosed herein can be readily used to isolate the complete gene from filamentous fungi, in particular *A. niger* which in turn can easily be subjected to further sequence analyses thereby identifying sequencing errors.

Unless otherwise indicated, all nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

The person skilled in the art is capable of identifying such erroneously identified bases and knows how to correct for such errors.

Nucleic acid fragments, probes and primers

A nucleic acid molecule according to the invention may comprise only a portion or a fragment of the nucleic acid sequence shown in a sequence selected from the group

consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114, for example a fragment which can be used as a probe or primer or a fragment encoding a portion of a protease protein. The nucleotide sequence determined from the cloning of the protease gene and cDNA

5 allows for the generation of probes and primers designed for use in identifying and/or cloning other protease family members, as well as protease homologues from other species. The probe/primer typically comprises substantially purified oligonucleotide which typically comprises a region of nucleotide sequence that hybridizes preferably under highly stringent conditions to at least about 12 or 15, preferably about 18 or 20,

10 preferably about 22 or 25, more preferably about 30, 35, 40, 45, 50, 55, 60, 65, or 75 or more consecutive nucleotides of a nucleotide sequence shown in a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 or of a functional equivalent thereof.

15

Probes based on the protease nucleotide sequences can be used to detect transcripts or genomic protease sequences encoding the same or homologous proteins for instance in other organisms. In preferred embodiments, the probe further comprises a label group attached thereto, e.g., the label group can be a radioisotope, a fluorescent

20 compound, an enzyme, or an enzyme cofactor. Such probes can also be used as part of a diagnostic test kit for identifying cells which express a protease protein.

Identity & homology

25 The terms "homology" or "percent identity" are used interchangeably herein. For the purpose of this invention, it is defined here that in order to determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a

30 second amino or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the

35 number of identical positions shared by the sequences (i.e., % identity = number of identical positions/total number of positions (i.e. overlapping positions) x 100).

Preferably, the two sequences are the same length.

- The skilled person will be aware of the fact that several different computer programs are available to determine the homology between two sequences. For instance, a
- 5 comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch (J. Mol. Biol. (48):444-453 (1970)) algorithm which has been incorporated into the GAP program in the GCG software package (available
- 10 at <http://www.gcg.com>), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. The skilled person will appreciate that all these different parameters will yield slightly different results but that the overall percentage identity of two sequences is not significantly altered when using different algorithms.
- 15 In yet another embodiment, the percent identity between two nucleotide sequences is determined using the GAP program in the GCG software package (available at <http://www.gcg.com>), using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. In another embodiment, the percent identity two amino acid or nucleotide sequence is determined using the algorithm of E.
- 20 Meyers and W. Miller (CABIOS, 4:11-17 (1989)) which has been incorporated into the ALIGN program (version 2.0) (available at <http://vega.igh.cnrs.fr/bin/align-guess.cgi>), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4.
- 25 The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the NBLAST and XBLAST programs (version 2.0) of Altschul, et al. (1990) J. Mol. Biol. 215:403—10. BLAST nucleotide searches can be performed with the NBLAST
- 30 program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to protease nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to protease protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as
- 35 described in Altschul et al., (1997) Nucleic Acids Res. 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the

respective programs (e.g., XBLAST and NBLAST) can be used. See
<http://www.ncbi.nlm.nih.gov>.

Hybridisation

5

As used herein, the term "hybridizing" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least about 50%, at least about 60%, at least about 70%, more preferably at least about 80%, even more preferably at least about 85% to 90%, more preferably at least 95% homologous to
10 each other typically remain hybridized to each other.

A preferred, non-limiting example of such hybridization conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45 °C, followed by one or more washes in 1 X SSC, 0.1 % SDS at 50 °C, preferably at 55 °C, preferably at 60 °C and
15 even more preferably at 65 °C.

Highly stringent conditions include, for example, hybridizing at 68 °C in 5x SSC/5x Denhardt's solution/1.0% SDS and washing in 0.2x SSC/0.1% SDS at room temperature. Alternatively washing may be performed at 42 °C.

20

The skilled artisan will know which conditions to apply for stringent and highly stringent hybridisation conditions. Additional guidance regarding such conditions is readily available in the art, for example, in Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, N.Y.; and Ausubel et al. (eds.), 1995, Current Protocols in Molecular Biology, (John Wiley & Sons, N.Y.).
25

Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of mRNAs), or to a complementary stretch of T (or U) residues, would not be included in a polynucleotide of the invention used to specifically hybridize
30 to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule contain a poly (A) stretch or the complement thereof (e.g., practically any double-standed cDNA clone).

Obtaining full length DNA from other organisms

35

In a typical approach, cDNA libraries constructed from other organisms, e.g.

filamentous fungi, in particular from the species *Aspergillus* can be screened.

For example, *Aspergillus* strains can be screened for homologous protease polynucleotides by Northern blot analysis. Upon detection of transcripts homologous to
5 polynucleotides according to the invention, cDNA libraries can be constructed from RNA isolated from the appropriate strain, utilizing standard techniques well known to those of skill in the art. Alternatively, a total genomic DNA library can be screened using a probe hybridisable to a protease polynucleotide according to the invention.

10 Homologous gene sequences can be isolated, for example, by performing PCR using two oligonucleotide primers or two degenerate oligonucleotide primer pools designed on the basis of nucleotide sequences as taught herein.

The template for the reaction can be cDNA obtained by reverse transcription of mRNA
15 prepared from strains known or suspected to express a polynucleotide according to the invention. The PCR product can be subcloned and sequenced to ensure that the amplified sequences represent the sequences of a new protease nucleic acid sequence, or a functional equivalent thereof.

20 The PCR fragment can then be used to isolate a full length cDNA clone by a variety of known methods. For example, the amplified fragment can be labeled and used to screen a bacteriophage or cosmid cDNA library. Alternatively, the labeled fragment can be used to screen a genomic library.

25 PCR technology also can be used to isolate full length cDNA sequences from other organisms. For example, RNA can be isolated, following standard procedures, from an appropriate cellular or tissue source. A reverse transcription reaction can be performed on the RNA using an oligonucleotide primer specific for the most 5' end of the amplified fragment for the priming of first strand synthesis.

30 The resulting RNA/DNA hybrid can then be "tailed" (e.g., with guanines) using a standard terminal transferase reaction, the hybrid can be digested with RNase H, and second strand synthesis can then be primed (e.g., with a poly-C primer). Thus, cDNA sequences upstream of the amplified fragment can easily be isolated. For a review of
35 useful cloning strategies, see e.g., Sambrook et al., supra; and Ausubel et al., supra.

Vectors

Another aspect of the invention pertains to vectors, preferably expression vectors, containing a nucleic acid encoding a protease protein or a functional equivalent thereof.

- 5 As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are
- 10 capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the
- 15 expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. The terms "plasmid" and "vector" can be used interchangeably herein as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression
- 20 vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

- The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which
- 25 means that the recombinant expression vector includes one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operatively linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows
- 30 for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (e.g., polyadenylation signal). Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in*
- 35 *Enzymology* 185, Academic Press, San Diego, CA (1990). Regulatory sequences include those which direct constitutive expression of a nucleotide sequence in many

- types of host cells and those which direct expression of the nucleotide sequence only in a certain host cell (e.g. tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce proteins or peptides, encoded by nucleic acids as described herein (e.g. protease proteins, mutant forms of protease proteins, fragments, variants or functional equivalents thereof, fusion proteins, etc.).
- 10 The recombinant expression vectors of the invention can be designed for expression of protease proteins in prokaryotic or eukaryotic cells. For example, protease proteins can be expressed in bacterial cells such as *E. coli*, insect cells (using baculovirus expression vectors) yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example using T7 promoter regulatory sequences and T7 polymerase.

Expression vectors useful in the present invention include chromosomal-, episomal- and virus-derived vectors e.g., vectors derived from bacterial plasmids, bacteriophage, yeast episome, yeast chromosomal elements, viruses such as baculoviruses, papova viruses, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids.

- 25 The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli* lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled person. In a specific embodiment, promoters are preferred that are capable of directing a high expression level of proteases in filamentous fungi. Such promoters are known in the art. The expression constructs may contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will include a translation initiating AUG at the beginning and a termination codon appropriately positioned at the end of the polypeptide to be translated.

Vector DNA can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (e.g., DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, transduction, infection, lipofection, cationic lipid-mediated transfection or electroporation. Suitable methods for transforming or transfecting host cells can be found in Sambrook, et al. (*Molecular Cloning: A Laboratory Manual*, 2nd, ed. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989), Davis et al., *Basic Methods in Molecular Biology* (1986) and other laboratory manuals.

For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a protease protein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (e.g. cells that have incorporated the selectable marker gene will survive, while the other cells die).

Expression of proteins in prokaryotes is often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion proteins. Fusion vectors add a number of amino acids to a protein encoded therein, e.g. to the amino terminus of the recombinant protein. Such fusion vectors typically serve three purposes: 1) to increase expression of recombinant protein; 2) to increase the solubility of the recombinant protein; and 3) to aid in the purification of the recombinant protein by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant protein to enable separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Such enzymes, and their cognate recognition sequences, include Factor Xa,

thrombin and enterokinase.

As indicated, the expression vectors will preferably contain selectable markers. Such markers include dihydrofolate reductase or neomycin resistance for eukarotic cell culture and tetracycline or ampicilling resistance for culturing in *E. coli* and other bacteria. Representative examples of appropriate host include bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium*; fungal cells, such as yeast; insect cells such as *Drosophila* S2 and *Spodoptera Sf9*; animal cells such as CHO, COS and Bowes melanoma; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria are pQE70, pQE60 and PQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16A, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia. Among preferred eukaryotic vectors are PWLNEO, pSV2CAT, pOG44, pZT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Among known bacterial promoters for use in the present invention include *E. coli* lacI and lacZ promoters, the T3 and T7 promoters, the gpt promoter, the lambda PR, PL promoters and the trp promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus ("RSV"), and metallothionein promoters, such as the mouse metallothionein-I promoter.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act to increase transcriptional activity of a promoter in a given host cell-type. Examples of enhancers include the SV40 enhancer, which is located on the late side of the replication origin at bp 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion

signal may be incorporated into the expressed polypeptide. The signals may be endogenous to the polypeptide or they may be heterologous signals.

5 The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals but also additional heterologous functional regions. Thus, for instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate
10 purification.

Polypeptides according to the invention

The invention provides an isolated polypeptide having an amino acid sequence
15 selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171, an amino acid sequence obtainable by expressing a polynucleotide according to the invention or in a preferred embodiment of a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 in an appropriate host, as well as an amino acid sequence obtainable by expressing a polynucleotide sequences selected from the group
20 consisting of SEQ ID NO: 58 to SEQ ID NO: 114 in an appropriate host. Also, a peptide or polypeptide comprising a functional equivalent of the above polypeptides is comprised within the present invention. The above polypeptides are collectively comprised in the term "polypeptides according to the invention".

25 The terms "peptide" and "oligopeptide" are considered synonymous (as is commonly recognized) and each term can be used interchangeably as the context requires to indicate a chain of at least two amino acids coupled by peptidyl linkages. The word "polypeptide" is used herein for chains containing more than seven amino acid residues. All oligopeptide and polypeptide formulas or sequences herein are written
30 from left to right and in the direction from amino terminus to carboxy terminus. The one-letter code of amino acids used herein is commonly known in the art and can be found in Sambrook, et al. (*Molecular Cloning: A Laboratory Manual*, 2nd, ed. Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989)

35

By "isolated" polypeptide or protein is intended a polypeptide or protein removed from

its native environment. For example, recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for purpose of the invention as are native or recombinant polypeptides which have been substantially purified by any suitable technique such as, for example, the single-step purification method disclosed
5 in Smith and Johnson, Gene 67:31-40 (1988).

The protease according to the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography,
10 phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. For analytical purposes most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

15 Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be
20 glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes.

Moreover, a protein according to the invention may be a precursor protein such as a
25 zymogen, a hybrid protein, a protein obtained as a pro sequence or pre-pro sequence, or any other type of immature form.

Protein fragments

30 The invention also features biologically active fragments of the polypeptides according to the invention.

Biologically active fragments of a polypeptide of the invention include polypeptides comprising amino acid sequences sufficiently identical to or derived from the amino
35 acid sequence of the protease protein (e.g., the amino acid sequence of a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171), which

include fewer amino acids than the full length protein, and exhibit at least one biological activity of the corresponding full-length protein. Typically, biologically active fragments comprise a domain or motif with at least one activity of the protease protein. A biologically active fragment of a protein of the invention can be a polypeptide which is, for example, 10, 25, 50, 100 or more amino acids in length. Moreover, other biologically active portions, in which other regions of the protein are deleted, can be prepared by recombinant techniques and evaluated for one or more of the biological activities of the native form of a polypeptide of the invention.

- 10 The invention also features nucleic acid fragments which encode the above biologically active fragments of the protease protein.

Fusion proteins

- 15 The proteins of the present invention or functional equivalents thereof, e.g., biologically active portions thereof, can be operatively linked to a non-protease polypeptide (e.g., heterologous amino acid sequences) to form fusion proteins. As used herein, a protease "chimeric protein" or "fusion protein" comprises a protease polypeptide operatively linked to a non-protease polypeptide. A "protease polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a polypeptide sequence according to the invention, whereas a "non-protease polypeptide" refers to a polypeptide having an amino acid sequence corresponding to a protein which is not substantially homologous to a protein according to the invention, e.g., a protein which is different from the protease protein and which is derived from the same or a different organism. Within a protease fusion protein the protease polypeptide can correspond to all or a portion of a protein according to the invention. In a preferred embodiment, a protease fusion protein comprises at least one biologically active fragment of a protein according to the invention. In another preferred embodiment, a protease fusion protein comprises at least two biologically active portions of a protein according to the invention. Within the fusion protein, the term "operatively linked" is intended to indicate that the protease polypeptide and the non-protease polypeptide are fused in-frame to each other. The non-protease polypeptide can be fused to the N-terminus or C-terminus of the protease polypeptide.
- 35 For example, in one embodiment, the fusion protein is a GST-protease fusion protein in which the protease sequences are fused to the C-terminus of the GST sequences.

- Such fusion proteins can facilitate the purification of recombinant protease. In another embodiment, the fusion protein is a protease protein containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian and Yeast host cells), expression and/or secretion of protease can be increased through use of a
- 5 heterologous signal sequence.
- In another example, the gp67 secretory sequence of the baculovirus envelope protein can be used as a heterologous signal sequence (*Current Protocols in Molecular Biology*, Ausubel et al., eds., John Wiley & Sons, 1992). Other examples of eukaryotic heterologous signal sequences include the secretory sequences of melittin and human
- 10 placental alkaline phosphatase (Stratagene; La Jolla, California). In yet another example, useful prokaryotic heterologous signal sequences include the phoA secretory signal (Sambrook et al., *supra*) and the protein A secretory signal (Pharmacia Biotech; Piscataway, New Jersey).
- 15 A signal sequence can be used to facilitate secretion and isolation of a protein or polypeptide of the invention. Signal sequences are typically characterized by a core of hydrophobic amino acids which are generally cleaved from the mature protein during secretion in one or more cleavage events. Such signal peptides contain processing sites that allow cleavage of the signal sequence from the mature proteins as they pass
- 20 through the secretory pathway. The signal sequence directs secretion of the protein, such as from a eukaryotic host into which the expression vector is transformed, and the signal sequence is subsequently or concurrently cleaved. The protein can then be readily purified from the extracellular medium by art recognized methods. Alternatively, the signal sequence can be linked to the protein of interest using a sequence which
- 25 facilitates purification, such as with a GST domain. Thus, for instance, the sequence encoding the polypeptide may be fused to a marker sequence, such as a sequence encoding a peptide, which facilitates purification of the fused polypeptide. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qiagen, Inc.), among
- 30 others, many of which are commercially available. As described in *Gentz et al, Proc. Natl. Acad. Sci. USA 86:821-824 (1989)*, for instance, hexa-histidine provides for convenient purification of the fusion protein. The HA tag is another peptide useful for purification which corresponds to an epitope derived of influenza hemagglutinin protein, which has been described by Wilson *et al.*, *Cell 37:767 (1984)*, for instance.
- 35 Preferably, a protease chimeric or fusion protein of the invention is produced by

standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, 5 filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene 10 fragments which can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for example, *Current Protocols in Molecular Biology*, eds. Ausubel *et al.* John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g. a GST polypeptide). A protease-encoding nucleic acid can be cloned into such an expression vector such that 15 the fusion moiety is linked in-frame to the protease protein.

Functional equivalents

The terms "functional equivalents" and "functional variants" are used interchangeably 20 herein. Functional equivalents of a DNA according to the invention are isolated DNA fragments that encode a polypeptide that exhibits a particular function of an *A. niger* protease as defined herein. A functional equivalent of a polypeptide according to the invention is a polypeptide that exhibits at least one function of an *A. niger* protease as defined herein.

25

Functional protein or polypeptide equivalents may contain only conservative substitutions of one or more amino acids of a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 or substitutions, insertions or deletions of non-essential amino acids. Accordingly, a non-essential amino acid is a 30 residue that can be altered in a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 without substantially altering the biological function. For example, amino acid residues that are conserved among the protease proteins of the present invention, are predicted to be particularly unamenable to alteration. Furthermore, amino acids conserved among the protease proteins according to the 35 present invention and other proteases are not likely to be amenable to alteration.

The term "conservative substitution" is intended to mean that a substitution in which the amino acid residue is replaced with an amino acid residue having a similar side chain.

These families are known in the art and include amino acids with basic side chains (e.g. lysine, arginine and histidine), acidic side chains (e.g. aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), non-polar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

10

Functional nucleic acid equivalents may typically contain silent mutations or mutations that do not alter the biological function of encoded polypeptide. Accordingly, the invention provides nucleic acid molecules encoding protease proteins that contain changes in amino acid residues that are not essential for a particular biological activity.

15

Such protease proteins differ in amino acid sequence from a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 yet retain at least one biological activity. In one embodiment the isolated nucleic acid molecule comprises a nucleotide sequence encoding a protein, wherein the protein comprises a substantially homologous amino acid sequence of at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous to the amino acid sequence shown in a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171.

20

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J.U. et al., Science 247:1306-1310 (1990) wherein the authors indicate that there are two main approaches for studying the tolerance of an amino acid sequence to change. The first method relies on the process of evolution, in which mutations are either accepted or rejected by natural selection. The second approach uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene and selects or screens to identify sequences that maintain functionality. As the authors state, these studies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which changes are likely to be permissive at a certain position of the protein. For example, most buried amino acid residues require non-polar side chains, whereas few features of surface side chains are generally conserved. Other such phenotypically silent substitutions are described in Bowie et al, supra, and the references cited therein.

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An isolated nucleic acid molecule encoding a protease protein homologous to the protein selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 can be created by introducing one or more nucleotide substitutions, additions or deletions
5 into the coding nucleotide sequences according to a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 such that one or more amino acid substitutions, deletions or insertions are introduced into the encoded protein. Such mutations may be introduced by standard techniques, such as site-directed
10 mutagenesis and PCR-mediated mutagenesis.

The term "functional equivalents" also encompasses orthologues of the *A. niger* protease protein. Orthologues of the *A. niger* protease protein are proteins that can be isolated from other strains or species and possess a similar or identical biological
15 activity. Such orthologues can readily be identified as comprising an amino acid sequence that is substantially homologous to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171.

As defined herein, the term "substantially homologous" refers to a first amino acid or
20 nucleotide sequence which contains a sufficient or minimum number of identical or equivalent (e.g., with similar side chain) amino acids or nucleotides to a second amino acid or nucleotide sequence such that the first and the second amino acid or nucleotide sequences have a common domain. For example, amino acid or nucleotide sequences which contain a common domain having about 60%, preferably 65%, more preferably
25 70%, even more preferably 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identity or more are defined herein as sufficiently identical.

Also, nucleic acids encoding other protease family members, which thus have a nucleotide sequence that differs from a sequence selected from the group consisting of
30 SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114, are within the scope of the invention. Moreover, nucleic acids encoding protease proteins from different species which thus have a nucleotide sequence which differs from a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting
35 of SEQ ID NO: 58 to SEQ ID NO: 114 are within the scope of the invention.

Nucleic acid molecules corresponding to variants (e.g. natural allelic variants) and homologues of the protease DNA of the invention can be isolated based on their homology to the protease nucleic acids disclosed herein using the cDNAs disclosed herein or a suitable fragment thereof, as a hybridisation probe according to standard
5 hybridisation techniques preferably under highly stringent hybridisation conditions.

In addition to naturally occurring allelic variants of the protease sequence, the skilled person will recognise that changes can be introduced by mutation into the nucleotide sequences of a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ
10 ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 thereby leading to changes in the amino acid sequence of the protease protein without substantially altering the function of the protease protein.

In another aspect of the invention, improved protease proteins are provided. Improved
15 protease proteins are proteins wherein at least one biological activity is improved. Such proteins may be obtained by randomly introducing mutations along all or part of the protease coding sequence, such as by saturation mutagenesis, and the resulting mutants can be expressed recombinantly and screened for biological activity. For instance, the art provides for standard assays for measuring the enzymatic activity of
20 proteases and thus improved proteins may easily be selected.

In a preferred embodiment the protease protein has an amino acid sequence according to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171. In another embodiment, the protease polypeptide is substantially homologous to
25 the amino acid sequence according to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 and retains at least one biological activity of a polypeptide according to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171, yet differs in amino acid sequence due to natural variation or mutagenesis as described above.

30 In a further preferred embodiment, the protease protein has an amino acid sequence encoded by an isolated nucleic acid fragment capable of hybridising to a nucleic acid according to a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ
35 ID NO: 114, preferably under highly stringent hybridisation conditions.

Accordingly, the protease protein is a protein which comprises an amino acid sequence at least about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more homologous to the amino acid sequence shown in a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 and retains at least one
5 functional activity of the polypeptide according to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171.

Functional equivalents of a protein according to the invention can also be identified e.g. by screening combinatorial libraries of mutants, e.g. truncation mutants, of the protein
10 of the invention for protease activity. In one embodiment, a variegated library of variants is generated by combinatorial mutagenesis at the nucleic acid level. A variegated library of variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential protein sequences is expressible as individual polypeptides, or
15 alternatively, as a set of larger fusion proteins (e.g., for phage display). There are a variety of methods that can be used to produce libraries of potential variants of the polypeptides of the invention from a degenerate oligonucleotide sequence. Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang (1983) Tetrahedron 39:3; Itakura et al. (1984) Annu. Rev. Biochem. 53:323; Itakura et al. (1984) Science 198:1056; Ike et al. (1983) Nucleic Acid Res. 11:477).
20

In addition, libraries of fragments of the coding sequence of a polypeptide of the invention can be used to generate a variegated population of polypeptides for screening a subsequent selection of variants. For example, a library of coding
25 sequence fragments can be generated by treating a double stranded PCR fragment of the coding sequence of interest with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by
30 treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal and internal fragments of various sizes of the protein of interest.

Several techniques are known in the art for screening gene products of combinatorial
35 libraries made by point mutations of truncation, and for screening cDNA libraries for gene products having a selected property. The most widely used techniques, which are

amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the
5 vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify variants of a protein of the invention (Arkin and Yourvan (1992) Proc. Natl. Acad. Sci. USA 89:7811-7815; Delgrave et al. (1993) Protein Engineering 6(3):327-331).

10

In addition to the protease gene sequence shown in a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57, it will be apparent for the person skilled in the art that DNA sequence polymorphisms that may lead to changes in the amino acid sequence of the protease protein may exist within a given population. Such
15 genetic polymorphisms may exist in cells from different populations or within a population due to natural allelic variation. Allelic variants may also include functional equivalents.

Fragments of a polynucleotide according to the invention may also comprise
20 polynucleotides not encoding functional polypeptides. Such polynucleotides may function as probes or primers for a PCR reaction. Such polynucleotides may also be useful when it is desired to abolish the functional activity of a protease in a particular organism (knock-out mutants).

25 Nucleic acids according to the invention irrespective of whether they encode functional or non-functional polypeptides, can be used as hybridization probes or polymerase chain reaction (PCR) primers. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having a protease activity include, inter alia,
(1) isolating the gene encoding the protease protein, or allelic variants thereof from a
30 cDNA library e.g. from other organisms than *A. niger*; (2) in situ hybridization (e.g. FISH) to metaphase chromosomal spreads to provide precise chromosomal location of the protease gene as described in Verma et al., *Human Chromosomes: a Manual of Basic Techniques*, Pergamon Press, New York (1988); (3) Northern blot analysis for detecting expression of protease mRNA in specific tissues and/or cells and 4) probes
35 and primers that can be used as a diagnostic tool to analyse the presence of a nucleic acid hybridisable to the protease probe in a given biological (e.g. tissue) sample.

Also encompassed by the invention is a method of obtaining a functional equivalent of a protease gene or cDNA. Such a method entails obtaining a labelled probe that includes an isolated nucleic acid which encodes all or a portion of the sequence
5 according to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 or a variant thereof; screening a nucleic acid fragment library with the labelled probe under conditions that allow hybridisation of the probe to nucleic acid fragments in the library, thereby forming nucleic acid duplexes, and preparing a full-length gene sequence from the nucleic acid fragments in any labelled duplex to obtain
10 a gene related to the protease gene.

In one embodiment, a protease nucleic acid of the invention is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more homologous to a nucleic acid sequence shown in a sequence selected from the group
15 consisting of SEQ ID NO: 1 to SEQ ID NO: 57, a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 or the complement thereof.

In another preferred embodiment a protease polypeptide of the invention is at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%,
20 99%, or more homologous to the amino acid sequence shown in a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171.

Host cells

25 In another embodiment, the invention features cells, e.g., transformed host cells or recombinant host cells that contain a nucleic acid encompassed by the invention. A "transformed cell" or "recombinant cell" is a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a nucleic acid according to the invention. Both prokaryotic and eukaryotic cells are included, e.g.,
30 bacteria, fungi, yeast, and the like, especially preferred are cells from filamentous fungi, in particular *Aspergillus niger*.

A host cell can be chosen that modulates the expression of the inserted sequences, or modifies and processes the gene product in a specific, desired fashion. Such
35 modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may facilitate optimal functioning of the protein.

Various host cells have characteristic and specific mechanisms for post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems familiar to those of skill in the art of molecular biology and/or microbiology can be chosen to ensure the desired and correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such host cells are well known in the art.

Host cells also include, but are not limited to, mammalian cell lines such as CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, WI38, and choroid plexus cell lines.

If desired, the polypeptides according to the invention can be produced by a stably-transfected cell line. A number of vectors suitable for stable transfection of mammalian cells are available to the public, methods for constructing such cell lines are also publicly known, e.g., in Ausubel et al. (supra):

Antibodies

The invention further features antibodies, such as monoclonal or polyclonal antibodies, that specifically bind protease proteins according to the invention.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')₂ fragments) which are capable of specifically binding to protease protein. Fab and F(ab')₂ fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983)). Thus, these fragments are preferred.

The antibodies of the present invention may be prepared by any of a variety of methods. For example, cells expressing the protease protein or an antigenic fragment thereof can be administered to an animal in order to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of protease protein is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal

antisera of greater specific activity.

- In the most preferred method, the antibodies of the present invention are monoclonal antibodies (or protease protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology (Kohler *et al.*, *Nature* 256:495 (1975);
- 5 Kohler *et al.*, *Eur. J. Immunol.* 6:511 (1976); Hammerling *et al.*, In: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, N.Y., pp. 563-681 (1981)). In general, such procedures involve immunizing an animal (preferably a mouse) with a protease protein antigen or, with a protease protein expressing cell. The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma
- 10 cell line may be employed in accordance with the present invention; however, it is preferably to employ the parent myeloma cell line (SP₂O), available from the American Type Culture Collection, Rockville, Maryland. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands *et al.* (*Gastro-enterology* 80:225-232 (1981)). The hybridoma cells
- 15 obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the protease protein antigen. In general, the polypeptides can be coupled to a carrier protein, such as KtH, as described in Ausubel *et al.*, supra, mixed with an adjuvant, and injected into a host mammal.
- 20 In particular, various host animals can be immunized by injection of a polypeptide of interest. Examples of suitable host animals include rabbits, mice, guinea pigs, and rats. Various adjuvants can be used to increase the immunological response, depending on the host species, including but not limited to Freund's (complete and incomplete), adjuvant mineral gels such as aluminum hydroxide, surface active substances such as
- 25 lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, dinitrophenol, BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of the immunized animals.
- 30 Such antibodies can be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD, and any subclass thereof. The hybridomas producing the mAbs of this invention can be cultivated *in vitro* or *in vivo*.
- Once produced, polyclonal or monoclonal antibodies are tested for specific recognition
- 35 of an protease polypeptide or functional equivalent thereof in an immunoassay, such as a Western blot or immunoprecipitation analysis using standard techniques, e.g., as

described in Ausubel et al., supra. Antibodies that specifically bind to protease proteins or functional equivalents thereof are useful in the invention. For example, such antibodies can be used in an immunoassay to detect protease in pathogenic or non-pathogenic strains of *Aspergillus* (e.g., in *Aspergillus* extracts).

5

Preferably, antibodies of the invention are produced using fragments of the protease polypeptides that appear likely to be antigenic, by criteria such as high frequency of charged residues. For example, such fragments may be generated by standard techniques of PCR, and then cloned into the pGEX expression vector (Ausubel et al., supra). Fusion proteins may then be expressed in *E. coli* and purified using a glutathione agarose affinity matrix as described in Ausubel, et al., supra. If desired, several (e.g., two or three) fusions can be generated for each protein, and each fusion can be injected into at least two rabbits. Antisera can be raised by injections in a series, typically including at least three booster injections. Typically, the antisera are checked for their ability to immunoprecipitate a recombinant protease polypeptide or functional equivalents thereof whereas unrelated proteins may serve as a control for the specificity of the immune reaction.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent 4,946,778 and 4,704,692) can be adapted to produce single chain antibodies against a protease polypeptide or functional equivalents thereof. Kits for generating and screening phage display libraries are commercially available e.g. from Pharmacia.

Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223, 409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 20791; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs et al. (1991) *Bio/Technology* 9:1370-1372; Hay et al. (1992) *Hum. Antibod. Hybridomas* 3:81-85; Huse et al. (1989) *Science* 246:1275-1281; Griffiths et al. (1993) *EMBO J.* 12:725-734.

Polyclonal and monoclonal antibodies that specifically bind protease polypeptides of functional equivalents thereof can be used, for example, to detect expression of a protease gene or a functional equivalent thereof e.g. in another strain of *Aspergillus*.

For example, protease polypeptide can be readily detected in conventional immunoassays of *Aspergillus* cells or extracts. Examples of suitable assays include, without limitation, Western blotting, ELISAs, radioimmune assays, and the like.

- 5 By "specifically binds" is meant that an antibody recognizes and binds a particular antigen, e.g., a protease polypeptide, but does not substantially recognize and bind other unrelated molecules in a sample.

- 10 Antibodies can be purified, for example, by affinity chromatography methods in which the polypeptide antigen is immobilized on a resin.

- An antibody directed against a polypeptide of the invention (e.g., monoclonal antibody) can be used to isolate the polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, such an antibody can be used to
15 detect the protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the polypeptide. The antibodies can also be used diagnostically to monitor protein levels in cells or tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen or in the diagnosis of Aspergillosis..

20

- Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline
25 phosphatase, β -galactosidase, or acetylcholinesterase; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable
30 radioactive materials include ^{125}I , ^{131}I , ^{35}S or ^3H .

- Preferred epitopes encompassed by the antigenic peptide are regions that are located on the surface of the protein, e.g., hydrophilic regions. Hydrophobicity plots of the proteins of the invention can be used to identify hydrophilic regions.
- 35 The antigenic peptide of a protein of the invention comprises at least 7 (preferably 10, 15, 20, or 30) contiguous amino acid residues of the amino acid sequence of a

sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 and encompasses an epitope of the protein such that an antibody raised against the peptide forms a specific immune complex with the protein.

- Preferred epitopes encompassed by the antigenic peptide are regions of protease that
- 5 are located on the surface of the protein, e.g., hydrophilic regions, hydrophobic regions, alpha regions, beta regions, coil regions, turn regions and flexible regions.

Immunoassays

- 10 Qualitative or quantitative determination of a polypeptide according to the present invention in a biological sample can occur using any art-known method. Antibody-based techniques provide special advantages for assaying specific polypeptide levels in a biological sample.
- 15 In these, the specific recognition is provided by the primary antibody (polyclonal or monoclonal) but the secondary detection system can utilize fluorescent, enzyme, or other conjugated secondary antibodies. As a result, an immunocomplex is obtained.

- Accordingly, the invention provides a method for diagnosing whether a certain
- 20 organism is infected with *Aspergillus* comprising the steps of:
- Isolating a biological sample from said organism suspected to be infected with *Aspergillus*,
 - reacting said biological sample with an antibody according to the invention,
 - determining whether immunocomplexes are formed.

25

Tissues can also be extracted, e.g., with urea and neutral detergent, for the liberation of protein for Western-blot or dot/slot assay. This technique can also be applied to body fluids.

- 30 Other antibody-based methods useful for detecting protease gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). For example, protease-specific monoclonal antibodies can be used both as an immunoabsorbent and as an enzyme-labeled probe to detect and quantify the protease protein. The amount of protease protein present in the sample
- 35 can be calculated by reference to the amount present in a standard preparation using a

linear regression computer algorithm. In another ELISA assay, two distinct specific monoclonal antibodies can be used to detect protease protein in a biological fluid. In this assay, one of the antibodies is used as the immuno-absorbent and the other as the enzyme-labeled probe.

5

The above techniques may be conducted essentially as a "one-step" or "two-step" assay. The "one-step" assay involves contacting protease protein with immobilized antibody and, without washing, contacting the mixture with the labeled antibody. The "two-step" assay involves washing before contacting the mixture with the labeled
10 antibody. Other conventional methods may also be employed as suitable. It is usually desirable to immobilize one component of the assay system on a support, thereby allowing other components of the system to be brought into contact with the component and readily removed from the sample.

15 Suitable enzyme labels include, for example, those from the oxidase group, which catalyze the production of hydrogen peroxide by reacting with substrate. Activity of an oxidase label may be assayed by measuring the concentration of hydrogen peroxide formed by the enzyme-labelled antibody/substrate reaction.

20 Besides enzymes, other suitable labels include radioisotopes, such as iodine (^{125}I , ^{127}I), carbon (^{14}C), sulphur (^{35}S), tritium (^3H), indium (^{112}In), and technetium ($^{99\text{m}}\text{Tc}$), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Specific binding of a test compound to a protease polypeptide can be detected, for
25 example, in vitro by reversibly or irreversibly immobilizing the protease polypeptide on a substrate, e.g., the surface of a well of a 96-well polystyrene microtitre plate. Methods for immobilizing polypeptides and other small molecules are well known in the art. For example, the microtitre plates can be coated with a protease polypeptide by adding the polypeptide in a solution (typically, at a concentration of 0.05 to 1 mg/ml in a volume of
30 1-100 μl) to each well, and incubating the plates at room temperature to 37 $^{\circ}\text{C}$ for 0.1 to 36 hours. Polypeptides that are not bound to the plate can be removed by shaking the excess solution from the plate, and then washing the plate (once or repeatedly) with water or a buffer. Typically, the polypeptide is contained in water or a buffer. The plate is then washed with a buffer that lacks the bound polypeptide. To block the free
35 protein-binding sites on the plates, the plates are blocked with a protein that is unrelated to the bound polypeptide. For example, 300 μl of bovine serum albumin

(BSA) at a concentration of 2 mg/ml in Tris-HCl is suitable. Suitable substrates include those substrates that contain a defined cross-linking chemistry (e.g., plastic substrates, such as polystyrene, styrene, or polypropylene substrates from Corning Costar Corp. (Cambridge, MA), for example) . If desired, a beaded particle, e.g., beaded agarose or
5 beaded sepharose, can be used as the substrate.

Binding of the test compound to the polypeptides according to the invention can be detected by any of a variety of artknown methods. For example, a specific antibody can be used in an immunoassay. If desired, the antibody can be labeled (e.g., fluorescently
10 or with a radioisotope) and detected directly (see, e.g., West and McMahon, J. Cell Biol. 74:264, 1977). Alternatively, a second antibody can be used for detection (e.g., a labeled antibody that binds the Fc portion of an anti-AN97 antibody). In an alternative detection method, the protease polypeptide is labeled, and the label is detected (e.g.,
15 by labeling a protease polypeptide with a radioisotope, fluorophore, chromophore, or the like). In still another method, the protease polypeptide is produced as a fusion protein with a protein that can be detected optically, e.g., green fluorescent protein (which can be detected under UV light). In an alternative method, the protease polypeptide can be covalently attached to or fused with an enzyme having a detectable enzymatic activity, such as horse radish peroxidase, alkaline phosphatase, a-
20 galactosidase, or glucose oxidase. Genes encoding all of these enzymes have been cloned and are readily available for use by those of skill in the art. If desired, the fusion protein can include an antigen, and such an antigen can be detected and measured with a polyclonal or monoclonal antibody using conventional methods. Suitable antigens include enzymes (e.g., horse radish peroxidase, alkaline phosphatase, and a-
25 galactosidase) and non-enzymatic polypeptides (e.g., serum proteins, such as BSA and globulins, and milk proteins, such as caseins).

Epitopes, antigens and immunogens.

30 In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. These immunogenic
35 epitopes are believed to be confined to a few loci on the molecule. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic

epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen, H. M. et al., *Proc. Natl. Acad. Sci. USA* 81:3998-4002 (1984).

- 5 As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G. et al., *Science* 219:660-666 (1984). Peptides
- 10 capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Peptides that are extremely hydrophobic and those of six or fewer residues generally are ineffective at inducing
- 15 antibodies that bind to the mimicked protein; longer, soluble peptides, especially those containing proline residues, usually are effective. Sutcliffe et al., *supra* ?. For instance, 18 of 20 peptides designed according to these guidelines, containing 8-39 residues covering 75% of the sequence of the influenza virus hemagglutinin HA1 polypeptide chain, induced antibodies that reacted with the HA1 protein or intact virus; and 12/12
- 20 peptides from the MuLV polymerase and 18/18 from the rabies glycoprotein induced antibodies that precipitated the respective proteins.

- Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a
- 25 polypeptide of the invention. Thus, a high proportion of hybridomas obtained by fusion of spleen cells from donors immunized with an antigen epitope-bearing peptide generally secrete antibody reactive with the native protein. Sutcliffe et al., *supra*, at 663. The antibodies raised by antigenic epitope bearing peptides or polypeptides are useful to detect the mimicked protein, and antibodies to different peptides may be used
- 30 for tracking the fate of various regions of a protein precursor which undergoes posttranslation processing. The peptides and anti-peptide antibodies may be used in a variety of qualitative or quantitative assays for the mimicked protein, for instance in competition assays since it has been shown that even short peptides (e.g., about 9 amino acids) can bind and displace the larger peptides in immunoprecipitation assays.
- 35 See, for instance, Wilson, I.A. et al., *Cell* 37:767-778 at 777 (1984). The anti-peptide antibodies of the invention also are useful for purification of the mimicked protein, for

instance, by adsorption chromatography using methods well known in the art.

Antigenic epitope-bearing peptides and polypeptides of the invention designed according to the above guidelines preferably contain a sequence of at least seven,
5 more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of a polypeptide of the invention, containing about 30 to about 50 amino acids, or any length up to and including the entire amino acid sequence of a
10 polypeptide of the invention, also are considered epitope-bearing peptides or polypeptides of the invention and also are useful for inducing antibodies that react with the mimicked protein. Preferably, the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (i.e., the sequence includes relatively hydrophilic residues and highly hydrophobic sequences
15 are preferably avoided); and sequences containing proline residues are particularly preferred.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means for making peptides or polypeptides including recombinant
20 means using nucleic acid molecules of the invention. For instance, a short epitope-bearing amino acid sequence may be fused to a larger polypeptide which acts as a carrier during recombinant production and purification, as well as during immunization to produce anti-peptide antibodies.

Epitope-bearing peptides also may be synthesized using known methods of chemical synthesis. For instance, Houghten has described a simple method for synthesis of large numbers of peptides, such as 10-20 mg of 248 different 13 residue peptides representing single amino acid variants of a segment of the HAI polypeptide which were prepared and characterized (by ELISA-type binding studies) in less than four
25 weeks. Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten et al. (1986). In this procedure the individual resins for the solid-phase synthesis of various peptides are contained in separate solvent-permeable packets, enabling the optimal use of the many identical repetitive steps
30 involved in solid-phase methods.

A manual procedure allows 500-1000 or more syntheses to be conducted simultaneously. Houghten et al., *supra*, at 5134.

5 Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow, M. et al., *Proc. Natl. Acad. Sci. USA* 82:910-914; and Bittle, F.J. et al., *J. Gen. Virol.* 66:2347-2354 (1985).

10 Generally, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling of the peptide to a macromolecular carrier, such as keyhole limpet hemocyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine may be coupled to carrier using a linker such as maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carrier using a more general linking agent such as glutaraldehyde.

15 Animals such as rabbits, rats and mice are immunized with either free or carriercoupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 ug peptide or carrier protein and Freund's adjuvant. Several booster injections may be needed, for instance, at intervals of about two weeks, to
20 provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

25 Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. For instance, Geysen et al., 1984, *supra*, discloses a procedure for rapid concurrent synthesis on solid supports of
30 hundreds of peptides of sufficient purity to react in an enzyme-linked immunosorbent assay. Interaction of synthesized peptides with antibodies is then easily detected without removing them from the support. In this manner a peptide bearing an immunogenic epitope of a desired protein may be identified routinely by one of ordinary skill in the art. For instance, the immunologically important epitope in the coat protein of
35 foot-and-mouth disease virus was located by Geysen et al. with a resolution of seven amino acids by synthesis of an overlapping set of all 208 possible hexapeptides

covering the entire 213 amino acid sequence of the protein. Then, a complete replacement set of peptides in which all 20 amino acids were substituted in turn at every position within the epitope were synthesized, and the particular amino acids conferring specificity for the reaction with antibody were determined. Thus, peptide
5 analogs of the epitope-bearing peptides of the invention can be made routinely by this method. U.S. Patent No. 4,708,781 to Geysen (1987) further describes this method of identifying a peptide bearing an immunogenic epitope of a desired protein.

Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method
10 of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical
15 equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. et al. (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated
20 oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

Removal or reduction of protease activity

25 The present invention also relates to methods for producing a mutant cell of a parent cell, which comprises disrupting or deleting a nucleic acid sequence encoding the protease or a control sequence thereof, which results in the mutant cell producing less of the protease than the parent cell.

30 The construction of strains which have reduced protease activity may be conveniently accomplished by modification or inactivation of a nucleic acid sequence necessary for expression of the protease activity in the cell. The nucleic acid sequence to be modified or inactivated may be, for example, a nucleic acid sequence encoding the
35 protease or a part thereof essential for exhibiting protease activity, or the nucleic acid sequence may have a regulatory function required for the expression of the protease

from the coding sequence of the nucleic acid sequence. An example of such a regulatory or control sequence may be a promoter sequence or a functional part thereof, i.e., a part which is sufficient for affecting expression of the protease. Other control sequences for possible modification include, but are not limited to, a leader, a polyadenylation sequence, a propeptide sequence, a signal sequence, and a termination site.

Modification or inactivation of the nucleic acid sequence may be performed by subjecting the cell to mutagenesis and selecting for cells in which the protease producing capability has been reduced or eliminated. The mutagenesis, which may be specific or random, may be performed, for example, by use of a suitable physical or chemical mutagenizing agent, by use of a suitable oligonucleotide, or by subjecting the DNA sequence to PCR generated mutagenesis. Furthermore, the mutagenesis may be performed by use of any combination of these mutagenizing agents.

Examples of a physical or chemical mutagenizing agent suitable for the present purpose include ultraviolet (UV) irradiation, hydroxylamine, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), O-methyl hydroxylamine, nitrous acid, ethyl methane sulphonate (EMS), sodium bisulphite, formic acid, and nucleotide analogues.

When such agents are used, the mutagenesis is typically performed by incubating the cell to be mutagenized in the presence of the mutagenizing agent of choice under suitable conditions, and selecting for cells exhibiting reduced or no expression of protease activity.

Modification or inactivation of production of a protease of the present invention may be accomplished by introduction, substitution, or removal of one or more nucleotides in the nucleic acid sequence encoding the protease or a regulatory element required for the transcription or translation thereof. For example, nucleotides may be inserted or removed so as to result in the introduction of a stop codon, the removal of the start codon, or a change of the open reading frame. Such modification or inactivation may be accomplished by site-directed mutagenesis or PCR generated mutagenesis in accordance with methods known in the art.

Although, in principle, the modification may be performed *in vivo*, i.e., directly on the cell expressing the nucleic acid sequence to be modified, it is preferred that the modification be performed *in vitro* as exemplified below.

An example of a convenient way to inactivate or reduce production by a host cell of

choice is based on techniques of gene replacement or gene interruption. For example, in the gene interruption method, a nucleic acid sequence corresponding to the endogenous gene or gene fragment of interest is mutagenized in vitro to produce a defective nucleic acid sequence which is then transformed into the host cell to produce
5 a defective gene. By homologous recombination, the defective nucleic acid sequence replaces the endogenous gene or gene fragment. It may be desirable that the defective gene or gene fragment also encodes a marker which may be used for selection of transformants in which the gene encoding the protease has been modified or destroyed.

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Alternatively, modification or inactivation of the nucleic acid sequence encoding a protease of the present invention may be performed by established anti-sense techniques using a nucleotide sequence complementary to the protease encoding sequence. More specifically, production of the protease by a cell may be reduced or
15 eliminated by introducing a nucleotide sequence complementary to the nucleic acid sequence encoding the protease which may be transcribed in the cell and is capable of hybridizing to the protease mRNA produced in the cell. Under conditions allowing the complementary antisense nucleotide sequence to hybridize to the protease mRNA, the amount of protease translated is thus reduced or eliminated.

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It is preferred that the cell to be modified in accordance with the methods of the present invention is of microbial origin, for example, a fungal strain which is suitable for the production of desired protein products, either homologous or heterologous to the cell.

25 The present invention further relates to a mutant cell of a parent cell which comprises a disruption or deletion of a nucleic acid sequence encoding the protease or a control sequence thereof, which results in the mutant cell producing less of the protease than the parent cell.

30 The protease-deficient mutant cells so created are particularly useful as host cells for the expression of homologous and/or heterologous polypeptides. Therefore, the present invention further relates to methods for producing a homologous or heterologous polypeptide comprising (a) culturing the mutant cell under conditions conducive for production of the polypeptide; and (b) recovering the polypeptide. In the
35 present context, the term "heterologous polypeptides" is defined herein as polypeptides which are not native to the host cell, a native protein in which modifications have been

made to alter the native sequence, or a native protein whose expression is quantitatively altered as a result of a manipulation of the host cell by recombinant DNA techniques.

- 5 The methods of the present invention for producing an essentially protease-free product is of particular interest in the production of eukaryotic polypeptides, in particular fungal proteins such as enzymes. The protease-deficient cells may also be used to express heterologous proteins of interest for the food industry, or of pharmaceutical interest.

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Use of proteases in industrial processes

- The invention also relates to the use of the protease according to the invention in a selected number of industrial and pharmaceutical processes. Despite the long term
15 experience obtained with these processes, the protease according to the invention features a number of significant advantages over the enzymes currently used. Depending on the specific application, these advantages can include aspects like lower production costs, higher specificity towards the substrate, less antigenic, less undesirable side activities, higher yields when produced in a suitable microorganism,
20 more suitable pH and temperature ranges, better tastes of the final product as well as food grade and kosher aspects.

- In large scale industrial applications aimed at food or feed production, proteolytic enzymes are commonly used to improve aspects like protein solubility, extraction
25 yields, viscosity or taste, texture, nutritional value, minimalisation of antigenicity or antinutritional factors, colour or functionality as well as processing aspects like filterability of the proteinaceous raw material. In these applications the proteinaceous raw material can be of animal or vegetable origin and examples include vegetable proteins such as soy protein, wheat gluten, rape seed protein, pea protein, alfalfa protein, sunflower
30 protein, fabaceous bean protein, cotton or sesame seed protein, maize protein, barley protein, sorghum protein, potato protein, rice protein, coffee proteins, and animal derived protein such as milk protein (e.g. casein, whey protein), egg white, fish protein, meat protein including gelatin, collagen, blood protein (e.g. haemoglobin), hair, feathers and fish meal.

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An important aspect of the proteases according to the invention is that they cover a

whole range of pH and temperature optima which are ideally suited for a variety of applications. For example many large scale processes benefit from relatively high processing temperatures of 50 degrees C or higher to control the risks of microbial infections. Several proteases according to the invention comply with this demand but at the same time exhibit no extreme heat stabilities so that they resist attempts to inactivate the enzyme by an additional heat treatment. The latter feature allows production routes that yield final products free of residual proteolytic activity. Similarly many feed and food products have slightly acidic pH values so that for their processing proteases with acidic or near neutral pH optima are preferred. A protease according to the invention complies with this requirement as well.

The specificity of endoproteases is usually defined in terms of preferential cleavages of bonds between the carboxyl of the amino acid residue in position P1 and the amino group of the residue in position P1' respectively. The preference may be conditioned predominantly either by P1 (e.g. positively charged residues in substrates for trypsin), by P1' (e.g. hydrophobic residues in cleavages by thermolysin) or by both P1 and P2 (e.g. specific cleavages between two positively charged residues by adrenal medulla serine endoprotease). In some cases more distant residues may determine the cleavage preference, e.g. P2 for streptococcal peptidase A. Some residues are known to influence cleavages negatively; it is well known that bonds with proline in position P1' are resistant to the action of many proteases. Most endoproteases cleave preferentially either in a hydrophobic environment or in the proximity of negatively charged residues. For example, industrially available endoproteases like chymotrypsin (obtained from bovine pancreas) or subtilisin, neutral metallo endoprotease or thermolysin (all obtained from *Bacillus* species) tend to favour cleavage "behind" hydrophobic amino acids like -Phe, -Leu and -Tyr. Other industrially available endoproteases are trypsin (obtained from bovine pancreas) preferring cleavage behind -Arg and -Lys and papain (a complex mixture of various enzymes including proteases obtained from papaya fruits) preferring cleavage behind -Arg.

In contrast, peptide bonds formed by small sized residues such as Ala, Gly, Ser, Thre as well as Ile and Pro are poor substrates (Keil, B et al.; *Protein Seq Data Anal* (1993) 5; 401-407). This situation has a profound implications for the pharmaceutical, the food and beverages, the agro and even the chemical industry. A protease according to the invention exhibits uncommon cleavage preferences.

The exopeptidases act only near the ends of polypeptide chains. Those acting at a free N-terminus liberate a single amino acid residue (socalled aminopeptidases) or a dipeptide or a tripeptide (socalled dipeptidyl-peptidases and tripeptidyl-peptidases) Those acting at a free C-terminus liberate a single residu (socalled carboxypeptidases) or a dipeptide (socalled peptidyl-dipeptidases) The carboxypeptidases are allocated to three groups on the basis of catalytic mechanism i.e. serine-type carboxypeptidases, metallocarboxypeptidases and cystein-type carboxypeptidases. Other exopeptidases are specific for dipeptides (socalled dipeptidases) or are able to cleave peptide linkages other than those of alpha-carboxyl or alpha- amino groups (socalled omega peptidases). Examples of such new omega peptidases are the pyroglutamyl-peptidase and the acylaminoacyl-peptidase as identified in the present invention (see Table1, genes 18 and 45 respectively).

Typical examples of industrial application which depend on the use of pure endoproteases and in which the protease according to the invention can be expected to deliver a superior performance include the processing of materials of vegetable or animal origin. These processing steps can be aimed at modifying a large array of characteristics of either the crude material or the (partially) purified protein fraction. For example, these processing steps can be aimed at maximising product solubilities, filterabilities, separabilities, protein extraction yields and digestibilities or minimising toxicities, off-tastes and viscosities. Furthermore the treatment can be directed at altering physico-chemical characteristics of the crude material or the purified (or partially purified) protein. These advantages apply not only if the endoprotease according to the invention is applied as a processing aid in industrial applications but also if applied as an active enzyme component in animal feed. Specifically the endoprotease according to the invention can be applied as bread improver in the bakery industry, e.g. to retard the staling of bread or to diminishing the viscosity of doughs. Or the endoprotease can be used in the beer and wine industry to prevent or to minimise the formation of undesirable protein hazes. Alternatively it can be used in the beer industry to optimise the protein extraction yields of cereals used in the preparation of the wort. Furthermore, it can also be advantageously used in the dairy industry as a milk clotting agent with superior characteristics or to optimise the texturising, foaming or setting characteristics of various milk components. Another application in the dairy industry is the use of the new protease in the preparation of Enzyme Modified Cheeses (EMC's).

- Moreover, various proteinaceous substrates can be subjected to an endoprotease according to the invention, usually in combination with other proteolytic enzymes to obtain hydrolysates for medical or non- medical applications. Here the endoprotease according to the invention is surprisingly effective in achieving a complete hydrolysis of the proteinaceous substrate so that even protease resistant parts are fully hydrolysed, the endoprotease is also surprisingly active in minimising the allergenicity of the final hydrolysate or in suppressing the formation of bitter off-tastes.
- 10 More specifically the endoprotease according to the invention is characterised by its preference for cleaving proteins at unusual peptide bonds, especially with the small size amino acid residues of Ala, Gly, Ser and Thr, or the residues Ile and Pro in either the P1 or the P1' position (Keil, B et al.; Protein Seq Data Anal (1993) 5; 401-407). As the result those fractions of the proteinaceous starting materials that resist hydrolysis
- 15 upon using prior art endoproteases, can be dissolved and hydrolysed using the endoprotease according to the invention. Non limiting examples of such protease resistant fractions include so called extensins in plant materials and collagen, gelatin but also specific milk components in material of animal origin.
- 20 Various feedstuffs such as e.g. soybeans contain trypsin inhibitors. These proteins inhibit trypsin activity in the GI-tract of e.g. pigs and poultry. This trypsin inhibiting activity results in sub-optimal protein digestibility in these animals resulting in increased waste production and poor economics. This problem may partly be overcome by
- 25 toasting soybeans at high temperatures. Two different types of trypsin inhibitors have been identified in soybeans, i.e. the Bowman-Birk type trypsin inhibitors and the Kunitz type trypsin inhibitors.

This invention now provides an alternative way to degrade trypsin inhibiting activity over toasting, in that it provides a cysteine proteases (EC 3.4.22, table 1) capable of

30 cleaving at Leucine176-Aspartate177 peptide bond near the carboxyl-terminus of the Kunitz type trypsin inhibitor (as reviewed by Wilson (1988) in CRC Critical Reviews in Biotechnology 8 (3): 197-216). This results in inactivation of this trypsin inhibitor in soybean. It was surprisingly found that the cysteine proteases secreted by the fungus *Aspergillus niger* fulfilled these criteria far better than similar enzymes derived from

35 other organisms.

Proteases are also widely used in the art of cheese-making. In the production of cheese it is necessary to coagulate the cheese milk to be able to separate the cheese matters e.g. casein from the whey. Several milk coagulating enzymes, also referred to as coagulants, have been described and include (bovine) chymosin, bovine pepsin, porcine pepsin as well as microbial enzymes like *Rhizomucor miehei* protease, *Rhizomucor pusillus* protease and *Cryptonectria parasitica* protease. Chymosin can be obtained from calf stomachs but can also be produced microbially by for example *Kluyveromyces lactis*. All these enzymes are characterized by having specificity for the peptide bond between residue 105 (phenylalanine) and residue 106 (methionine) or the bond adjacent to that in κ -casein. This means that by employing these enzymes in cheese making, the κ -casein is split at the junction between para- κ -casein and the macro-peptide moiety called glycomacropeptide (GMP) carrying the negative charges. When this occurs the macropeptide diffuses into the whey, its stabilizing effect on the solubility of the casein micelles is lost, and the casein micelles can start to aggregate once sufficient kappa-casein has been hydrolyzed. For further elaboration on the enzymatic coagulation of milk (e.g. D.G. Dalgleish in Advanced Dairy Chemistry vol.1 ed by P.F. Fox, Elsevier, London, 1992.

The currently available coagulants allow for a rather high yield of cheese, however, it should be realised that due to the enormous volumes of cheese produced, an increased yield in the order of magnitude of tenths of percent points may constitute a substantial economical advantage. Consequently there is a great need in the art for coagulants with an (even slightly) improved yield.

Coagulants are characterized by their high substrate specificity, which is, however, dependent on pH and temperature. In a typical cheese making process the pH will change from the initial pH 6.3 to lower pH values in the range of 4.5-5.5, the end-value depends on the conditions used during the cheese production process. Some coagulants are more sensitive to pH changes than others. The *Rhizomucor pusillus* protease for example is more sensitive to pH changes than chymosin. Besides pH, also other parameters like temperature and water content may affect the protease specificity. It is well known that most coagulants show a changing substrate specificity with changing pH, resulting in altered proteolytic activity in later stages of the cheese making process. It is also well known that coagulants differ in the extent of casein proteolysis; they may also show differences in the peptide patterns produced during

proteolysis. These are relevant factors during cheese ripening and may affect cheese properties like taste, flavor and texture. In some cases coagulants give rise to undesired effects like the formation of bitter tasting peptides or off-taste. In addition, changes in proteolytic specificity may lead to a reduction in yield. Pepsin, a well known component in many bovine chymosin preparations, is an example of a protease that gives rise to lower yields and taste effects as compared to pure chymosin. There is still a need for coagulants which give rise to new, improved cheese texture and taste. Such new coagulants result in the accelerated development of taste and texture profiles related to cheese aging, therewith providing a substantial economical benefit.

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It is well known that free amino acids are very important in taste and flavour generation. Especially the amino acids leucine, phenylalanine, methionine and valine play an important role in the generation of typical cheese taste and flavor components. The free amino acids are converted via fermentation by micro organisms that are added during the cheese manufacturing process into the actual flavor and taste generating compounds like methanediol, dimethyldisulphide, methylpropanoic acid and methylpropanal. Exo-peptidases play an important role in the generation of free amino acids. They can only be effective, however, when they are combined with an endo-protease of appropriate specificity. Appropriate combinations of exo- and endo-peptidases can be used in cheese making, resulting in the manufacture of cheeses with new and improved taste profiles.

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The enzymes according to the invention may be used to hydrolyze proteinaceous materials of animal origin such as whole milk, skim milk, casein, whey protein or mixtures of casein and whey protein. Such mixtures of casein and whey protein may be used, for example, in ratios similar to those found in human milk. Furthermore, the enzyme mixture according to the invention may be used to hydrolyze proteinaceous materials of plant origin such as, for example, wheat gluten malted or unmalted barley or other cereals used for making beer, soy milk, concentrates or isolates thereof, maize protein concentrates and isolates thereof, and rice proteins.

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Within the area of large scale industrial processes, some applications rely on the use of endoproteases only whereas in other applications combinations of endoproteases with exoproteases are essential. Typical examples which depend on the use of pure endoproteases and in which the protease according to the invention can deliver a

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superior performance include applications like the processing of soy or peas or cereals proteins aimed at minimising viscosities or optimising foaming or other physico-chemical characteristics, bread improvers in the bakery industry also aimed at diminishing the viscosity of doughs, processing aids in the beer and wine industry aimed at the prevention of protein hazes or optimising the extraction yields of cereals, feed additives in the bio industry aimed at enhancing intestinal absorption or modulating microbial activities in the gut, processing aids in the dairy industry aimed at optimising the clotting, foaming or setting characteristics of various milk components. Moreover, v For specific market segments proteins derived from milk or soy or collagen are exposed to proteases to produce so-called protein hydrolysates. Although the main outlets for these protein hydrolysates are infant formula and food products for hospitalised persons, products intended for persons with non-medical needs, such as athletes or people on a slimming diet form a rapidly growing segment. In all of these applications protein hydrolysates offer attractive advantages such as lowered allergenicities, facilitated gastro-intestinal uptake, less chemical deterioration of desirable amino acids like glutamine and cysteine and finally, absence of proteinaceous precipitations in acid beverages during prolonged storage periods. All these advantages can be combined if the hydrolysate is offered as a mixture of di- and tripeptides. However, currently all commercially available hydrolysates are produced by combining several endoproteases. The latter approach implies a non-uniform and incomplete degradation of the protein. To obtain the desired mixture of di- and tripeptides, a hydrolysis process involving a combination of various di- and tripeptidylpeptidases would be ideal. Unfortunately, only few of these enzymes from food grade and industrially acceptable microorganisms are known, let alone industrially available. According to the invention several of highly useful di- and tripeptidylpeptidases are economically obtainable in a relatively pure state. Preferred are those di- or tripeptidylpeptidases that exhibit a low selectivity towards the substrate to be cleaved, i.e. exhibit minimal amino acid residue cleavage preferences only. Preferred are combinations of those di- or tripeptidylpeptidases that hydrolyse high percentage of the naturally occurring peptide bonds. Despite this high activity to naturally occurring peptide bonds, a total hydrolysis to free amino acids is prevented by the nature of the di- and tripeptidylpeptidases. Also preferred are those di- or tripeptidylpeptidases that are optimally active between pH 4 to 8 and exhibit adequate temperature stability. Adequate temperature stability implies that at least 40%, preferably at least 60%, more preferably between 70 and 100% of the initial hydrolytic activity survives after heating the enzyme together with the substrate for 1 hour at 50

degrees C.

Although the process towards an efficient production of mixtures di-or tripeptides or di-and tripeptides hinges on the availability of the enzymes according to the invention, the first enzyme incubation with the proteinaceous substrate will usually be an endoprotease. Preferably an endoprotease with a broad spectrum endopeptidase suited for the situation, e.g. subtilisin (Delvolase from DSM), neutral metallo protease (Neutrase from NOVO) or thermolysin (Thermoase from Daiwa Kasei) for the near neutral conditions and pepsin or aspergillopepsin (e.g. Sumizyme AP from Shin Nihon, Japan) for the acidic conditions. Aim of this first digestion is to improve the solubility, to reduce the viscosity and to reduce the heat setting characteristics of the water/protein mixture. Furthermore this pretreatment with an endonuclease is essential to create enough starting points for the di- and tripeptidylpeptidases hereby accelerating the process of di- or tripeptide formation. Optionally a protease intended for debittering of the hydrolysate can be included in this stage of the process or later, together with the di-or tripeptidylpeptidases.

Main aim of the latter hydrolysates is to minimize the allergenicity of the product or to facilitate gastro-intestinal uptake. In the production of such hydrolysates the use of dipeptidyl- and tripeptidyl-peptidases is of special importance as these offer an efficient way for producing hydrolysates..

Other applications in these food and feed industries totally rely upon combinations of one or more endoprotease(s) with one or more exoprotease(s). Such combinations of an endoprotease with an exoprotease are typically used in industries to improve aspects like taste and colour of the final product. The reason for this is that the development of taste and colour is largely dependent upon the presence of free amino acids. Free amino acids can not only be obtained by exoproteases such as carboxypeptidases and aminopeptidases but also by peptidyl-dipeptidases. If combined with endoproteases or even dipeptidyl-or tripeptidyl-peptidases, carboxypeptidases, aminopeptidases and peptidyl-dipeptidases can create larger quantities of free amino acids in less time. However, in all of these processes an uncontrolled release of amino acids or even non-proteinaceous components should be avoided to minimise undesirable side reactions.

Though free amino acids as such, can elicit a number of taste impressions, these taste impressions are very basic (bitter, sweet, sour and "umami") and the amino acid

concentration required for perceiving these tastes are high. Despite these high threshold values, free amino acids are able to create major sensory effects at much lower concentration ranges through a number of flavour enhancing mechanisms. One of these mechanism involves the combination of free amino acids with sugars in so-called Maillard reactions. Compared with free amino acids, with these Maillard products overwhelmingly complex flavour and odour systems can develop with threshold values that are several orders of magnitude lower than those recorded for the free amino acids. Maillard products are formed at elevated temperatures usually during cooking, baking or roasting when preparing food or feed products. During these treatments both colour and a large array of aromas develop. In these reactions amino groups react with reducing compounds as a first step and ultimately leading to a whole family of reaction pathways. In foods or feeds the amino compounds involved are predominantly free amino acids which are released from the proteinaceous raw material by various proteases and the required reducing compounds primarily represent reducing sugars. The implication is that during the processing of the raw material undesired release of free amino acids and sugars should be avoided to minimise off tastes that could be generated during subsequent heating steps as e.g. during spray drying or sterilisation. The latter notion emphasises once more the benefits of superior purity and low in-use costs of the enzyme according to the invention.

Apart from Maillard reactions, amino acids can also undergo important chemical transitions at ambient temperatures. The latter type of transitions are enzyme dependent and are quite common in fermented foods such as beer, yogurt, cheese ripening and meat and wine maturation processes. In these fermentation processes, free amino acids are liberated from the raw materials used by the proteases added or by proteolytic enzyme activity from the raw material or the microbial starters used. During the maturation phase microbial metabolic activity then converts the free amino acids into derivatives with increased sensoric properties. For example, L-leucine, L-isoleucine and L-valine lead to the formation of valuable fusel alcohols like amylalcohols and isobutanol in beer fermentation. Similarly cheese volatiles such as methanethiol and dimethyldisulphide have been traced back to the occurrence of methionine in cheese as well as methylpropanoic acid and methylpropanal to valine. Finally the free amino acid glutamate and can create strong savoury enhancing effects because of its synergy with the breakdown products of RNA, so-called 5'-ribonucleotides. If combined with proper concentrations of 5'-ribonucleotides such as

5'-IMP and 5'-GMP, the detection threshold of the umami taste generated by glutamate is known to be lowered by almost two orders of magnitude.

In order to obtain pronounced and precise taste effects in all of these processes, the proteinaceous substrates should be hydrolysed using a combination of an endo- and an exoprotease, wherein at least one of the endo or exoprotease, preferably both the endo- and exoprotease, are pure and preferably selective towards a specific set of amino acid(s) or preferentially release the preferred amino acid(s). So preferred proteases are characterised by a high selectivity towards the amino acid sequences that can be cleaved which notion makes the enzyme category in *Aspergillus* known as "maturases" of particular importance.

Apart from the food and feed industries, proteases are also commonly applied by the chemical, pharmaceutical, diagnostic and personal care industries.

In the personal care industry proteases are used to create peptides which are added to a variety of products to improve aspects like skin feel, gloss or protection. Moreover there is a new tendency towards direct topical application of the protease. Very similar to the enzyme use in the leather industry, the prime aim in the latter application is to clean, dehair and soften the skin .

In the chemical and pharmaceutical industry proteases are being developed as valuable tools in producing costly ingredients or intermediates. In these industries proteases are not only used because of their hydrolytic capacity but also because of their capacity to synthesise peptides from natural or non-natural amino acids. The latter option is clearly demonstrated by the possibility to synthesize aspartame from its amino acid based building blocks by using an endoprotease like thermolysin.

Unlike the situation in the food and feed industry, the stereo- and regioselectivity of proteases are also considered important assets although unusual reaction conditions may be needed to accomplish the desired chemical transformation. Typical examples of the application of proteases in this industry include the use of endoproteases, aminopeptidases as well as carboxypeptidases in the production of various intermediates for drugs like insulin, antibiotics, renin and ACE-inhibitors An overview of such uses is presented in Industrial Biotransformations, A.Liese, K. Seelbach, C. Wandrey, Wiley-VCH; ISBN 3-527-30094-5.

In view of the desired specificities, stereo- and regioselectivities, the absence of side activities and resistance to unusual reaction conditions such as high solvent

concentrations, the improved performance of the protease according to the invention offers substantial advantages.

From a pharmaceutical point of view the role of proteases is illustrated by a substantial number of references in Martindale's, "The Extra Pharmacopoeia" (Pharmaceutical Press, London, UK). Moreover the important role of very specific proteases in regulating all kinds of biological processes is illustrated by the fact that many hormones become active only after the processing of an, mostly inactive, precursor molecule by such a very specific protease. Inhibitors active towards certain categories of such specific proteases have been implicated in the development of all kinds of new drugs. Therefore new and effective inhibitors for protease may now be identified using the sequences provided herein.

The entire disclosure of each document cited herein is hereby incorporated by reference

Table 1

SEQ ID number			Function of encoded protein	EC number
Gene	cDNA	Protein		
1	58	115	Pepsin A ₃	EC3.4.23.1
2	59	116	Metalloprotease	EC3.4.24.56
3	60	117	acylaminoacyl-peptidase	EC3.4.19.1
4	61	118	Tripeptidylaminopeptidase	EC3.4.14.-
5	62	119	serine carboxypeptidase	EC3.4.16.6
6	63	120	Serine endoprotease	EC3.4.21.-
7	64	121	Carboxypeptidase Y	EC3.4.16.5
8	65	122	aspergillopepsin II - hom	EC3.4.23.19
9	66	123	Tripeptidyl peptidase	EC3.4.14.9
10	67	124	Tripeptidyl peptidase	EC3.4.14.9
11	68	125	aspergillopepsin II - hom	EC3.4.23.19
12	69	126	Tripeptidyl peptidase	EC3.4.14.9
13	70	127	Metalloprotease	EC3.4.24.-
14	71	128	aspergillopepsin I	EC3.4.23.18
15	72	129	Pepsinogen E	EC3.4.23.25
16	73	130	aspergillopepsin I - hom	EC3.4.23.18
17	74	131	aspergillopepsin II	EC3.4.23.19
18	75	132	Pyro-Glu peptidase	EC3.4.19.3
19	76	133	dipeptidyl peptidase	EC3.4.14.2
20	77	134	Secr. aminopeptidase	EC3.4.11.10
21	78	135	alkaline D-peptidase	EC3.4.16.4
22	79	136	Carboxypeptidase	EC3.4.16.1
23	80	137	Carboxypeptidase	EC3.4.16.1
24	81	138	Carboxypeptidase-II	EC3.4.16.1
25	82	139	aspartic proteinase	EC3.4.23.-
26	83	140	Tripeptidyl peptidase	EC3.4.14.9
27	84	141	Carboxypeptidase	EC3.4.16.1
28	85	142	cysteine proteinase	EC3.4.22.-
29	86	143	Metallo-carboxypeptidase	EC3.4.17.-

SEQ ID number			Function of encoded protein	EC number
Gene	cDNA	Protein		
30	87	144	Subtilisin hom.	EC3.4.21.62
31	88	145	Carboxypeptidase Y	EC3.4.16.5
32	89	146	Metalloprotease	EC3.4.24.-
33	90	147	Carboxypeptidase Y	EC3.4.16.5
34	91	148	Metalloprotease	EC3.4.24.-
35	92	149	Tripeptidyl peptidase	EC3.4.14.9
36	93	150	Aspartic protease	EC3.4.23.24
37	94	151	Aspartic protease	EC3.4.23.24
38	95	152	Pepsin A ₃	EC3.4.23.1
39	96	153	Aspartic protease	EC3.4.23.24
40	97	154	Aspartic protease	EC3.4.23.24
41	98	155	Kex	EC3.4.21.61
42	99	156	Serine protease	EC3.4.21.-
43	100	157	Glutamyl endoprotease	EC3.4.21.82
44	101	158	aspergillopepsin II - hom	EC3.4.23.19
45	102	159	acylaminoacyl-peptidase	EC3.4.19.1
46	103	160	Tripeptidylaminopeptidase	EC3.4.14.-
47	104	161	serine carboxypeptidase	EC3.4.16.6
48	105	162	Gly-X carboxypeptidase	EC3.4.17.4
49	106	163	aspartic proteinase	EC3.4.23.-
50	107	164	Tripeptidyl peptidase	EC3.4.14.9
51	108	165	Carboxypeptidase-I	EC3.4.16.1
52	109	166	serine carboxypeptidase	EC3.4.16.6
53	110	167	serine carboxypeptidase	EC3.4.16.6
54	111	168	Secr. aminopeptidase	EC3.4.11.10
55	112	169	Prolyl endopeptidase	EC3.4.21.26
56	113	170	aspergillopepsin I - hom	EC3.4.23.18
57	114	171	Aminopeptidase	EC 3.4.11.-

EXAMPLES

Example 1

5 Assaying Proteolytic Activity and Specificity

Protease specificity may be explored by using various peptide substrates. Synthetic substrates are widely used to detect proteolytic enzymes in screening, in fermentation, during isolation, to assay enzyme activity, to determine enzyme concentrations, to investigate specificity and to explore interaction with inhibitors. Peptide p-nitroanilides
10 are preferably used to assay protease activity as the activity can be followed continuously and therefore allow for kinetic measurement. The cleavage of peptide p-nitroanilides can be followed by measuring the increase in adsorption at 410nm upon release of the 4-nitroanilide. Paranitroanilide substrates are generally used for serine and cysteine proteases. In addition peptide thioesters and 7-amino-p-methylcoumarin
15 peptide derivatives are used. Peptide thioesters are very sensitive substrates for serine and metalloproteases that exhibit relatively high turnover rate since the thioesterbond is easier to cleave than the amide bond. Cleavage of thioesters may be followed with a thiol reagent such 4,4-dithiopyridine (324nm) or 5,5-dithiobis 2-nitrobenzoic acid (405nm). The same increased turnover rate is usually observed for the cleavage of
20 ester bonds relative to amide bond. The most well known substrates to assay the esterase activity of proteases are p-nitrophenol derivatives. The release of p-nitrophenol can be monitored at different wavelength dependent on the pH that is used, eg around neutral pH a wavelength of 340nm is used while above pH 9 monitoring is done around 405nm. In addition the hydrolysis of esters can also be followed by titration using pH-stat equipment. In case of qualitative measurement of esterase activity pH sensitive
25 dyes can be applied.

As an alternative, peptides may be attached to a fluorescent leaving group. Proteolysis is accompanied by an increase in fluorescence when monitored at the appropriate
30 wavelengths. Peptidyl 2-naphtylamides and peptidyl 4-methyl-7-coumarylamides are commonly used. The release of for example 7-amino-4 methylcoumarin is measured using an excitation wavelength of 350nm and an emission wavelength of 460nm. The use of 7-amino-4 trifluoromethylcoumarin has the advantage of the leaving group being both chromogenic (absorbtion 380nm) as well as flourogenic (excitation 400nm,
35 emission 505nm). When it is essential that at both sides of the scissile bond an amino

acid is present, the introduction of a group that quenches the fluorescence might be useful. The general characteristics of such substrates is that the peptide sequence separates a fluorescent donor group from an acceptor group that acts as a quencher of of fluorescence. Cleavage of a peptide bond between the quenching group and the fluorophore will lead to substantial increase in fluorescence. Several donor-acceptor pairs have been reported, including o-aminobenzoic acid (Abz) as the donor and 2, 4 dinitrophenyl (Dnp) as the acceptor, 5-[(2'aminoethyl)-amino]naphtalenesulfonic acid (EDANS) as the donor and 4-[[4'-(dimethylamino)phenyl]azo]-benzoic acid (DABCYL) as the acceptor. The Abz/EDDnp represents a very convenient donor-acceptor pair since after total hydrolysis, the fluorescence increases by a factor 7 to 100 and the absorption spectrum of EDDnp does not change with pH. Moreover, the peptide sequence may contain up to 10 residues without loss of the quenching effect. As the size of the connecting peptides increases, the position of the scissile bond may become less specific. Therefore in addition to establishing whether proteolysis occurred, additional analysis of the products may be required. This may be done by analysing and separating the produced peptides by HPLC and determining the amino acid sequence of the fragments. In addition the peptide composition of the digest may be directly analysed by using combined HPLC / mass-spectroscopy technique.

20

Apart from using peptides of a defined sequence also synthetic peptide libraries can be used to study protease specificity. Peptides are synthesised by solid phase synthesis in random or semi-random fashion. E.g. Meldal et al. (PNAS USA 91,3314,1994) report the preparation of a family of protease substrates by starting with H-Lys(Abz)-resin, extending the resin with peptides to a length of six aminoacids, and finally coupling Tyr(NO₂) to the peptides. Each resin bead has a unique sequence and on treatment with the proteases the most susceptible becomes fluorescent as the Tyr(NO₂) containing peptide is released. Sequence analysis of the peptides on the susceptible will give information on the specificity of the protease.

30

Protease activity is usually expressed in units. Generally the international standard unit (IU) is defined as the amount of enzyme, which under defined conditions transfers one micromole of substrate per minute. Specifically with proteases the IU would relate to the hydrolysis of one micromole peptide bond per minute. However in the case of protease units deviations of the international definition are more rule than exception. Where with the model peptides, which are cleaved specifically at one bond the

35

- calculation of IU's is strait-forward, for proteinacious substrates where the protease can cleave at various positions to a various degree many deviating unit definition are used. Apart from a definition of the unit used, any hydrolysis experiment requires an adequate description of the conditions under which the units are measured. Such
- 5 conditions comprise e.g. the substrate concentration, the enzyme-substrate ratio, the pH and temperature. Typical assays for determining the specific activity of a proteases comprise a proteinacious substrate such as for example denaturated hemoglobin, insulin or casein. The polypeptide substrate is digested by a protease at fixed conditions during a fixed time interval. Undigested and large polypeptides are
- 10 precipitated with TCA and TCA soluble product is determined by measuring absorbance at 220 or 280nm, or by titrating the soluble peptides with folin reagent, ninhydrin, fluoro 2,4, dinitrobenzene/ dansylchloride, TNBS method or fluorescein. Instead of labeling the product after hydrolysis, also polypeptide substrates may be used which are already labeled by specific dyes or fluorophores such as for example
- 15 fluorescein. In addition standard methods of amino acid analysis may be applied using standard laboratory analyzers. In order to hget insight in the size distribution of the peptides generated by a protease, gel chromatography experiments may be performed. In addition to this HPLC using reverse phase techniques is applied in order to get better resolution of the peptide patterns generated by the protease.
- 20 The course of the hydrolysis of proteinacious substrates is usually expressed in the degree of hydrolysis or DH. In case pH-stat is used to follow the course of hydrolysis, DH can be derived from the base consumption during hydrolysis (Enzymatic Hydrolysis of Food Protein, J. Adler-Nissen, 1986, Elsevier Apllied Science Publishers LTD). The DH is related to various useful functional properties of the hydrolysate such as
- 25 solubility, emulsifying capacity, foaming and foam stability, whipping expansion, organoleptic quality. In addition taste is an important aspect of food grade hydrolysates. Bitterness can be a major problem in protein hydrolysates. Termination of the hydrolysis reaction may be done by changing the pH, heat inactivation, denaruring agents such as SDS, acetonitril etc.
- 30
- Polypeptides shown in Tabel 1 were expressed and at least partially purified according to standard procedures known in the art. They were analysed according to al least one of the methods described above and found to have the activities listed in Table 1.
- 35 Example 2

Direct determination of the k_{cat}/K_m ratio for protease substrates.

Synthetic substrates can be used to monitor the enzymatic activity during purification, to determine enzyme concentration, to determine inhibition constants or to investigate the substrate specificity. Determination of the k_{cat}/K_m ratio gives a measurement of the substrate specificity. It allows to compare the specificity of different substrates for a same enzyme or the comparison of hydrolysis rates with different enzymes cleaving the same substrate. This ratio has a unit of a second order rate constant and is then expressed as $1/(\text{concentration} \cdot \text{time})$. Substrates having a k_{cat}/K_m ratio in the range $10.5\text{--}10.6 \text{ M}^{-1} \cdot \text{sec}^{-1}$ are considered to be very good substrates i.e good affinity and rapid turn-over. However, some substrates may be very specific with k_{cat}/K_m values in the $10.4 \text{ M}^{-1} \cdot \text{sec}^{-1}$ range.

The k_{cat}/K_m ratio may be calculated after determination of individual parameters. In that case, K_m and V_m may be obtained from various linear plots (e.g Hanes or Cornish-Bowden method) or by a non-linear regression method. Knowing that $V_m = k_{cat} \cdot E_t$ (where E_t is the final active enzyme concentration then $k_{cat} = V_m/E_t$). Determination of the k_{cat}/K_m ratio by the previous method may be prevented when product or substrate inhibition occur, or when substrate precipitates at high concentration. It is however possible to obtain an accurate value of the k_{cat}/K_m ratio working under first-order conditions i.e at a substrate concentration far below the estimated K_m . In these conditions, the Michaelis-Menten equation: $v = (V_m \cdot S)/(K_m + S)$ becomes:

$$v = (V_m \cdot S)/K_m \text{ since } S \ll K_m$$

$$\text{or } v = (V_m/K_m) \cdot S = k_{obs} \cdot S = -dS/dt$$

which integrates as $\ln S = -k_{obs} \cdot t + \ln S_0$ where S_0 is the starting substrate concentration and S the substrate concentration at a given time. The velocity is proportionnal to the substrate concentration. In other words, the substrate hydrolysis obeys a first order process with k_{obs} as the first-order rate constant. $k_{obs} = V_m/K_m = (k_{cat} \cdot E_t)/K_m$ since $V_m = k_{cat} \cdot E_t$

A continuously recording of the substrate hydrolysis will allow the graphical determination of k_{obs} from the $\ln S$ vs time graph. The k_{cat}/K_m ratio is simply inferred from k_{obs} providing the active enzyme concentration is known:

$$k_{cat}/K_m = k_{obs}/E_t$$

Assay method: Use a starting substrate concentration far below the estimated K_m and a low enzyme concentration to allow the substrate hydrolysis to be recorded. You will obtain a first-order curve for the product generation:

After total hydrolysis of the substrate, the absorbance (or fluorescence units) of the

product will allow the accurate determination of S_0 , since $P_t = S_0 - k_{obs}t$. k_{obs} is determined from the slope of the $\ln S$ vs time graph or alternatively using a fitting software (Enzfitter, SigmaPlot...).

- 5 NB: Do not forget to calculate the substrate concentration for any given time from the product concentration ($S = S_0 - P$) since plotting P vs time would not provide the correct k_{obs} ($dP/dt = k_{obs}S$ does not integrate in the same way).

Alternatively, one can measure successive $t_{1/2}$ (half-time) from the product apparition curve since in a first order process:

$$t_{1/2} = \ln 2 / k_{obs} = 0.693 / k_{obs} \text{ then } k_{obs} = 0.693 / t_{1/2}$$

- 10 Using this method allows to check that you have a true first order decay (identical values for the successive $t_{1/2}$).

Example 3

Inactivating protease genes in *Aspergillus*

- 15 The most convenient way of inactivating protease genes in the genome of *Aspergillus* is the technique of gene replacement (also called "one step gene disruption"). The basics of this technique have been described by Rothstein RJ in Meth. Enzymol. 101, p202, 1983. Essentially the technique is based on homologous recombination of transformed DNA fragments with the genomic DNA of a fungal cell. Via double
- 20 crossover the gene to be inactivated is (partly) replaced by the DNA fragment with which the cell is transformed. Preferably the transformed DNA fragment contains a selectable marker gene for *Aspergillus niger*. Basically the manipulation of DNA and generation of a inactivation construct are done using general molecular biological techniques. First, genomic DNA is isolated from the *Aspergillus niger* strain that is later
- 25 on used for the inactivation of the protease gene. Genomic DNA of *A. niger* can be isolated by any of the techniques described, e.g. by the method described by de Graaff et al. (1988) Curr. Genet. 13, 315-321, and known to the person skilled in the art. This genomic DNA is used as template for amplification of the flanking regions of the protease gene by using the polymerase chain reaction (PCR; Sambrook et al. (1989)
- 30 Molecular cloning, a laboratory manual, 2nd edition, Cold Spring Harbor Laboratory Press, New York). With flanking regions is meant here the non-coding regions upstream and downstream of the protease gene that will be inactivated. Preferably the flanking regions should each be more than 1.0 kb in length.

- Two single stranded DNA oligonucleotides are used for the priming of the PCR
- 35 amplification of each flanking region. For the 5'-flanking region, one primer is homologous to a DNA sequence upstream of the start of the coding sequence of the

protease gene. Preferably the homologous region is located more than 1.0 kb upstream of the translation start site. The second primer is homologous to the complementary and inverse DNA sequence located immediately upstream of the coding sequence of the protease gene.

- 5 For the 3'-flanking region, one primer is homologous to the DNA sequence immediately downstream of the coding sequence of the protease gene. The second primer is homologous to a complementary and inverse DNA sequence located preferably more than 1.0 kb downstream of the coding sequence of the protease gene.
- The DNA sequence included in all primers and homologous to the *A. niger* genome
- 10 should be minimally 15 nucleotides in length, preferably more than 18 nucleotides in length. Most conveniently, all primers should contain a DNA sequence coding for the recognition site of suitable restriction enzymes upstream of the sequence that is homologous to the *A. niger* genome. These extra recognition sites facilitate the cloning process.
- 15 Both primers and the genomic DNA of *A. niger* are used in a PCR reaction under conditions known to those skilled in the art. The annealing temperature of the primers can be calculated from the part of the DNA sequence that is homologous to the *A. niger* genome. Both fragments containing the 5'-flanking region and the 3'-flanking region are cloned into a vector that can be propagated in *E. coli* using general
- 20 molecular biological techniques. A gene that can be used as selection marker in *Aspergillus niger* is then cloned in between the two flanking regions. Most conveniently the marker gene is under control of a promoter that comes to expression in *A. niger*, preferably an endogenous *A. niger* promoter. The orientation of the insertion of the marker gene is preferably in the same direction as the original protease gene. The final
- 25 inactivation fragment contains the 5'-flanking region, a selection marker gene preferably under control of a *A. niger* endogenous promoter, and the 3'-flanking region, all in this direction and orientation. DNA of the final construct is cloned into a vector that can be propagated in *E. coli*.
- The inactivation construct is digested with suitable restriction enzymes to remove the
- 30 *E. coli* vector sequences and the inactivation fragment is isolated using standard techniques (Sambrook et al. (1989) *Molecular cloning, a laboratory manual*, 2nd edition, Cold Spring Harbor Laboratory Press, New York). Finally *Aspergillus niger* is transformed with the inactivation fragment using a method described in literature, e.g. by the method described by Kusters-van Someren et al. (1991) *Curr. Genet.* 20, 293-
- 35 299. Transformed cells are selected by plating the transformation mixture on agar plates that are selective for growth of *Aspergillus niger* strains that do express the

- marker gene. After purification of the transformed *Aspergillus* strains by replica plating, a representative number of strains is analysed by Southern blotting using standard methods (Sambrook et al. (1989) Molecular cloning, a laboratory manual, 2nd edition, Cold Spring Harbor Laboratory Press, New York). Therefore, genomic DNA of
- 5 mycelium of transformed strains is isolated and digested with suitable restriction enzymes. Restriction fragments are separated using agarose gelelectrophoresis, blotted to nitrocellulose membranes and probed with a labeled fragment of the marker gene. Hybridization and washing is under stringent conditions. Strains that contain labeled restriction fragments of the correct length are considered correct.
- 10 Using this method *A. niger* strains can be selected with an inactivated protease gene of choice.

Example 4

Isolating proteases by ion exchange chromatography

- 15 Small quantities of the protease encoded by the nucleotide sequence as provided herein are obtained by constructing an expression plasmid containing the relevant DNA sequence, transforming an *A.niger* strain with this plasmid and growing the *A. niger* strain in a suitable medium. After collecting the broth free of contaminating cells, the protease sought can be purified.
- 20 To isolate the protease as encoded by the provided nucleotide sequence in an essentially pure form several strategies can be followed. All of these strategies have been adequately described in the relevant scientific literature (see for example the Protein Purification Handbook ,18-1132-29 Edition AA as published by Amersham Pharmacia Biotech, Uppsala, Sweden). A procedure which is applicable to purify
- 25 proteases from complex mixtures is provided hereunder. Essential is that a suitable assay is available that is selective towards the enzyme characteristics sought. For proteases typically a chromogenic, synthetic peptide substrate is used as described in Example 1. Such peptide substrates can be selective towards endoproteases, carboxypeptidases, aminopeptidases or omegapeptidases. In Example 11 the
- 30 selectivity towards a specific tripeptidylpeptidase is described. By choosing the right amino acid residues in the relevant synthetic peptide, proteases with the desired specificity can be selected.

- First it should be determined whether the protease is excreted into the medium,
- 35 depending on the expression system chosen to produce the protease, it may be excreted or contained in the cell. If the protease is excreted into the fermentation

- medium, the producing cells or fragments of these cells have to be removed by centrifugation or filtration and the resulting clear or clarified medium is the starting point for further purification. In those cases in which the protease sought is not excreted, the producing cells have to be disrupted to enable purification of the protease. In such
- 5 cases the collected cell mass is best ground with an abrasive, milled with beads, ultrasonicated or subjected to a French press or a Manton-Gaulin homogeniser and then filtered or centrifuged. In case the protease is hydrophobic or membrane bound, the addition of a non-ionic detergent to solubilise the protease before the filtering or centrifugation step may be necessary.
- 10 After the clarification step, a three phase purification strategy can be applied to obtain the unknown proteases in an essentially pure state. In all or some of these three phases addition of a detergent may be necessary.
- In the first or capture phase the target protease is isolated, partly purified and concentrated. During the subsequent intermediate purification phase most of the bulk
- 15 impurities are removed and in the final polishing phase trace amounts of remaining impurities of larger amounts of closely related substances are removed and the enzyme is dissolved in the desired buffer. Depending upon the nature and physical properties of the protease at hand, a person skilled in the art is capable of optimising the three phases using slightly modified versions of the different protein binding
- 20 materials and apply these under somewhat changed conditions. However, in all cases a selective analytical assay is indispensable as it will enable the continuous monitoring of the increasingly purified proteolytic activity. Analytical assays suitable for the purpose include the use of chromogenic peptide substrates as has been mentioned before.
- 25 In the first capturing phase of the purification a strong ion exchange resin of the anionic type is preferably used to apply the clarified and desalted enzyme containing medium. To guarantee binding of the desired proteolytic activity to the resin, three or four different pH values of medium and resin are tested under low conductivity conditions. In these tests the resin is always equilibrated with a buffer of the same pH value and
- 30 conductivity as the enzyme containing medium. The medium is then applied to the column under pH conditions which has been shown to allow adequate binding of the protease to the resin i.e. none of the desired enzymatic activity can be traced back in the run-through medium. Subsequently the desired enzymatic activity is eluted from the ion exchange resin using a continuous salt gradient which starts with the resin
- 35 equilibration buffer and ends with this buffer to which 1 mol/liter of NaCl has been added. Eluted fractions containing the desired activity according to the assay are

pooled and then prepared for an additional purification step. This additional purification step depends on the purity of the desired enzyme in the pooled fraction : if almost pure, an additional gel filtration step will prove to be adequate; if not almost pure, chromatography over a hydrophobic interaction resin is applied followed by a gel
5 filtration step.

Chromatography over a hydrophobic interaction resin is carried out by first increasing the salt content of the pooled fraction obtained from the ion exchange resin to 4mol/liter of NaCl and by removing any precipitate formed. If the resulting clear fraction doesnot
10 contain the desired activity, this activity is obviously present in the precipitate and can be recovered in an essentially pure state. If the resulting clear fraction still exhibits the desired activity in the assay, then the liquid is applied as such to a phenyl
sepharose resin (Pharmacia) equilibrated in this high salt buffer with an identical pH and conductivity. If the desired enzymatic activity binds to the phenyl sepharose resin,
15 the activity is eluted with a continuous gradient of decreasing salt content followed by a salt free wash and, if necessary, with a chaotropic agent. Like before those fractions from the gradient that exhibit activity in the assay are pooled and finally subjected to a
gelfiltration step. If the desired enzymatic activity doesnot bind to the phenyl sepharose resin, many of the contaminants will, so the desired proteolytic activity as present in the
20 void volume of the column requires only an additional ultrafiltration step to obtain the activity in a more concentrated form before applying it to the gelfiltration column. The gelfiltration column doesnot only remove trace contaminations but also brings the
enzyme in the buffer which is required by subsequent use.

25 Although this method is generally applicable for the isolation and purification of proteases according to the invention, a more specific isolation technique is described in Example 4. In that Example the isolation of an *Aspergillus* protease is described by using immobilised bacitracin, a peptide antibiotic known for its selective interaction with various types of proteases.

30

Example5.

Isolating proteases by affinity chromatography

An alternative method for purifying small quantities of protease is by affinity chromatography. To obtain the protease in a purified form, a 100 milliliter culture is
35 grown in a well aerated shake flask. After centrifugation to remove any non-soluble matter, the supernatant is applied to a 40 milliliter bacitracin-Sepharose column

equilibrated with 0.05 mol/litre sodium acetate pH 5.0. Proteases bound to the column are eluted using the acetate buffer supplemented with 1 mol/litre of NaCl and 10% (v/v) isopropanol (J.Appl.Biochem.,1983 pp420-428). Active fractions are collected, dialysed against distilled water and applied on a 20 milliliter bacitracin-Sepharose column, again
5 equilibrated with acetate buffer. As before, elution is carried out using the acetate buffer supplemented with NaCl and isopropanol. Active fractions, i.e. fractions displaying the activities sought, are collected, dialysed against a 5 millimol/litre acetate buffer pH 5.0 and then concentrated by means of ultrafiltration with a Amicon PM-10 membrane. To obtain the protease in an essentially pure state, the concentrated liquid
10 is chromatographed over a Superdex 75 column equilibrated with the 0.05 mol/litre sodium acetate buffer pH 5.0 and supplemented with 0.5 mol/litre NaCl. Further experiments carried out with the purified enzyme on PAGE may confirm if the molecular weight is in line with what can be expected on the basis of the available sequence data. Final confirmation can be obtained by carrying out a partial, N-terminal
15 amino acid analysis.

Example 6

Properties of a novel cysteine protease from *A. niger*.

In this Example *Aspergillus* gene nr 28 was cloned and overexpressed in *A. niger* as
20 described before. The enzyme obtained was purified according to procedures described in Example 4 and used to destroy trypsin inhibiting activity from soybeans under various conditions. As reference materials papain and bromelain were used. Bromelain was obtained from Sigma, papain was obtained from DSM Food Specialties Business Unit Beverage Ingredients, PO Box 1, 2600 MA Delft, the Netherlands. .

25 Trypsin inhibition was measured according to the method of Kakade, M.L., Rackis, J.J., McGhee, J.E. and Puski, G. (1974): J. Cereal Chemistry **51**: 376-382. Degradation of the substrate N-benzoyl-L-arginine-p-nitroaniline to N-benzoyl-L-arginine and p-nitroaniline was taken as a measure of trypsin activity. Trypsin was
30 obtained from British Drug Houses Ltd and was derived from cow's pancreas containing more than 0.54 Anson Units per gram of product. The Kunitz inhibitor for soybeans was also obtained from Sigma.

The trypsin inhibitor was pre-incubated at a concentration of 2 mg/ml with the above
35 mentioned cysteine protease enzymes at pH 3 in 50 mM Na-acetate buffer prior to measuring trypsin inhibition. Enzymes were added at a ratio of enzyme protein to

trypsin inhibitor of 1:100 (w/w). Albumin served as a negative control for the enzymes. Remaining trypsin activity was measured after incubation during 3 hours at 37°C. Results are shown in Table 2.

- 5 Table 2 . Effects of various cysteine proteases on the enzymatic inactivation of the Kunitz trypsin inhibitor from soybeans.

1	2	3	4	5
Enzyme tested	Remaining TI activity (%)	Remaining TI activity after pepsin treatment	Remaining TI activity after heat treatment at 75°C	Remaining TI activity after heat treatment at 90°C
Papain	25	55	78	95
Bromelain	30	62	86	99
<i>A.niger</i>	26	26	28	35
Albumin (control)	100	100	100	100

TI : Trypsin Inhibitor activity

- 10 Experiments were repeated in the presence of pepsin during the pre-incubation of cysteine proteases with the trypsin inhibitor. Pepsin was added at final concentration of 1.3 mg/ml. Results are shown in column 3.

- 15 Another series of experiments were conducted to check for heat stability. The cysteine proteases were incubated at 75 and 90°C during 5 minutes prior to the addition of these enzymes to the pre-incubation with the trypsin inhibitors. Results are shown in columns 4 and 5.

- 20 These results clearly demonstrate the superior activity of these novel cysteine proteases from *Aspergillus niger* over currently available cysteine proteases for the inactivation of trypsin inhibitors in animal feed.

Example 7.

Exo-peptidases promoting cheese ripening and cheese taste.

The amino-peptidases encoded by genes nr 20 and 54 (see Table 1) were overexpressed in *A.niger* according to methods described earlier. Purification of these enzymes was carried out according to procedures as described in Example 4. The activity of the purified enzyme samples was determined at pH7.2 in an aqueous phosphate buffer (50 mM) containing the para-nitro anilide derivative of a number of hydrophobic amino acids (3 mM) as the substrate. The conversion of the substrate by the amino peptidase was determined by monitoring the change in optical density at 400 nm as a result of substrate conversion, using a solution not containing the enzyme as the reference. Activity (A) was calculated as the change in OD per minute and expressed as e.g. Phe-AP, Leu-AP or Val-AP units, depending on the substrate used.

Normal cheese milk was inoculated with starter culture of the Delvo-tec™ DX 31 range (DSM Food Specialities Delft, The Netherlands) to obtain a Gouda-type cheese and coagulating was executed with an average dosis of coagulant (50 IMCU per liter of cheese milk). In addition, 25 Phe-units of each exo-protease was added to two experimental cheeses whereas the control did not contain either one of the exo-proteases. Cheese making parameters were used conform the procedure applied for semi-hard cheese for both cheeses. A difference was noted in terms of flavor and aroma development between the experimental cheeses and control cheese to such an extent that the experimental cheeses has obtained most of its organoleptical properties after three (3) weeks whereas the control cheese has obtained a similar qualification after six (6) weeks. The level of free amino acids after three weeks was shown to be twice as high in the experimental cheeses; after six weeks of ripening the levels were comparable again. Amino acid analysis was carried out according to the Picotag method of Waters (Milford MA, USA).

These data suggests that the product is ready for sale three weeks earlier without decreasing the keeping quality of the cheese. The organoleptic character of the experimental cheeses differed from the control to the extent that the bland cheese flavor with a slight tendency to bitterness of the control cheese was overcome in the experimental cheese in the presence of the amino-peptidase. The texture of the cheeses was found to be somewhat smoother as well.

Example 8

Novel specificity of a protease encoded by gene 55

As explained earlier, certain proteins can resist enzymatic hydrolysis as the result of specific amino acid compositions or specific tertiary structures. In such cases the quantity of peptides that can be solubilised from protease resistant proteins can be dramatically improved by using proteases exhibiting novel specificities.

Beta-casein is a protein with very limited tertiary structure but with an extraordinary high level of proline residues. Many proteases have difficulties in cleaving proline containing sequences so that the hydrolysis of beta-casein with commonly available proteases yields a hydrolysate that is relatively rich in large, protease-resistant peptides. The latter resistant peptides can attribute to a number of undesirable properties of the hydrolysate. For example, it is well known that these larger peptides have a relatively strong effect on allergenicity and bitterness. Moreover, these peptides withstand a further degradation into free amino acids so that in certain processes the occurrence of these large, protease resistant peptides are synonymous with yield losses. Therefore, the availability and use of proteases that are capable of cleaving the protease-resistant parts of the proteins, translate into serious technical and economical benefits.

Beta-casein represents one of the major casein fractions of bovine milk. The protein has been well characterised in terms of its amino acid sequence and is commercially available in an almost pure form. As such, beta-casein offers an excellent test substrate for studying the relationship between enzyme cleavage sites and the length of various peptides formed during enzyme hydrolysis.

This Example demonstrates that despite the broad spectrum cleavage character of the endoprotease subtilisin, the addition of a very specific enzyme like a prolyl endopeptidase as encoded by gene 55 (see Table 1) has a major impact on the size of the beta-casein fragments formed.

Beta-casein from bovine milk (lyophilised, essentially salt-free powder) with a minimum 90% beta-casein was obtained from Sigma. Subtilisin from *B.licheniformis* (Delvolase®, 560 000 DU per gram) was obtained from DSM Food Specialities (Seclin, France). The proline-specific endoprotease as encoded by gene 55 was overexpressed in *A. niger* and purified using procedures described in Example 4.

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Beta-casein powder was dissolved at a concentration of 10% (w/w) together with 0.1%

(w/w) Delvolase™ powder in a 0.1 mol/liter phosphate buffer pH7.0. After an incubation of 24 hours at 45°C in a shaking waterbath, the reaction was stopped by heating the solution for 15 minutes at 90°C. To one half of the solution (1ml containing 100milligrams of beta-casein) 100 microliter of the proline-specific protease was added
5 and the reaction was continued for another 24 hours at 45°C. After another heat shock at 90°C, samples of both the Delvolase™ and the Delvolase™ + proline-specific endoprotease treated beta-casein material were analysed by LC/MS equipment to study the precise peptide size distributions in the two samples.

10 LC/MS Analysis

HPLC using an ion trap mass spectrometer (Thermoquest™, Breda, the Netherlands) coupled to a P4000 pump (Thermoquest™, Breda, the Netherlands) was used in characterising the enzymatic protein hydrolysates produced by the inventive enzyme mixture. The peptides formed were separated using a PEPMAP C18 300A (MIC-15-03-
15 C18-PM, LC Packings, Amsterdam, The Netherlands) column in combination with a gradient of 0.1% formic acid in Milli Q water (Millipore, Bedford, MA, USA; Solution A) and 0.1% formic acid in acetonitrile (Solution B) for elution. The gradient started at 100% of Solution A and increased to 70% of solution B in 45 minutes and was kept at the latter ratio for another 5 minutes. The injection volume used was 50 microliters, the
20 flow rate was 50 microliter per minute and the column temperature was maintained at 30°C. The protein concentration of the injected sample was approx. 50 micrograms/milliliter.

Detailed information on the individual peptides was obtained by using the "scan
25 dependent" MS/MS algorithm which is a characteristic algorithm for an ion trap mass spectrometer. Full scan analysis was followed by zoom scan analysis for the determination of the charge state of the most intense ion in the full scan mass range. Subsequent MS/MS analysis of the latter ion resulted in partial peptide sequence information, which could be used for database searching using the SEQUEST
30 application from Xcalibur Bioworks (Thermoquest™, Breda, The Netherlands). Databanks used were extracted from the OWL.fasta databank, available at the NCBI (National Centre for Biotechnology informatics), containing the proteins of interest for the application used.

By using this technique as a screening method only peptides with a mass ranging from
35 approx. 400 to 2000 Daltons were considered suitable for further analysis by MS

sequencing.

Angiotensin (M=1295.6) was used to tune for optimal sensitivity in MS mode and for optimal fragmentation in MS/MS mode, performing constant infusion of 60 µg/ml, resulting in mainly doubly and triply charged species in MS mode, and an optimal collision energy of about 35 % in MS/MS mode.

In the sample digested with Delvolase alone, the LC/MS/MS analysis identified 40 peptides covering various parts of the beta-casein molecule. Together these peptides accounted for 79% of the total beta-casein sequence. Different retention times of the peptides on the C18 column could be traced back to peptide lengths ranging from 2 to 23 amino acid residues. Together < 15% of the peptides found were smaller than 6 amino acids. The sample digested with Delvolase™ and the proline-specific protease also generated a large number of identifiable peptides from beta-casein. Together these peptides covered > 50% of the total beta-casein protein sequence. In this sample the peptide size distribution was remarkably homogeneous, as the peptides ranged in length only between 2 and 6 residues. The results show that in the hydrolysate made with the proline-specific protease contain a large fraction of di-, tri-, up to 6 AA peptides, showing the distinct beneficial effect of the co-incubation with an endoprotease featuring an unusual specificity.. It is also clear from these experiments that the endoprotease according to gene 55 encodes an endoprotease that cleaves the peptide chain at the carboxyterminus of the proline residue.

Example 9

The selective release of specific amino acids to promote flavour formation.

Free amino acids like leucine and phenylalanine have not only been implicated in Maillard reactions but also as precursor for desirable aromas in various food fermentations. To promote the formation of such aromas in food fermentations or during the heating, roasting or baking phase of food, it would be advantageous to incorporate into these products a protein hydrolysate that contains relatively high levels of these specific amino acids in a free form. In this Example we describe the production of yeast extracts selectively enriched in leucine and phenylalanine. This enrichment is obtained by combining an endoprotease with a cleavage preference for a selected set of amino acid residues with an exoprotease favouring the release of a similar set of amino acid residues. The preference of the endoprotease should match with the preference of the exoprotease used. For example we have established that the

- aminopeptidases encoded by genes 20 and 54 (see Table1) feature a definite preference for releasing leucine and phenylalanine residues which matches with the cleavage preferences of thermolysin. The carboxypeptidases encoded by genes 23 and 24 have a preference for releasing arginine and lysine residues which matches the cleavage preferences of trypsin. Carboxypeptidase encoded by gene 5 features a highly unusual preference for releasing glycine which could be combined with certain endoproteases present in papaine. The carboxypeptidase encoded by gene 51 is capable of removing glutamate residues which matches the glutamate specific protease encoded by gene 43.
- 10 The endoprotease thermolysin (commercially available as Thermoase)C 180 from Daiwa Kasei KK (Osaka, Japan) is known to cleave peptide bonds at the amino terminal side of bulky, hydrophobic amino acids like Leu and Phe. To liberate the thus exposed amino acids from the newly formed peptides, we used the amino-peptidases encoded by genes nr 20 and 54 (see Table 1). These genes were overexpressed in
- 15 A.niger according to methods described earlier and purification of these enzymes was carried out according to procedures as described in Example 4.
- To release as much leucine and phenylalanine as possible without concomitant release of undesired amino acids with this combination of enzymes, it is evident that the conditions used during enzymatic hydrolysis should be carefully selected. Moreover,
- 20 the yeasts own endogeneous (and probably aspecific) proteases have to be inactivated. After a number of test incubations, a protocol was worked out that leads to a surprisingly selective and effective release of leucine and phenylalanine from the yeast proteins using these two new enzymes.
- 25 To inactivate the yeasts endogeneous proteases, the yeast suspension was kept for 5 minutes at 95 degrees C. Then the suspension was quickly cooled down to the required temperature and the pH was adjusted to 7.0 using 4N NaOH. The yeast, the thermolysin and one of the aminopeptidases were all incubated simultaneously under the following conditions. After the heat shock, the pH of the 2000 milliliters yeast
- 30 suspension was adjusted to 7.0 after which 680 milligrams of Thermoase were added and, after stirring, the purified aminopeptidase. The mixture was incubated with stirring at 50 degrees C for 3 hours and centrifuged. To stop all enzymatic activities the pH of the supernatant was adjusted to 4 and subjected to another heat treatment of 45 minutes at 95 degrees C. After another centrifugation a sample for amino acid analysis
- 35 was obtained from the supernatant. Precipitated or non-dissolved matter was removed by centrifugation for 15 minutes at 3500 rpm in an Hereaus Megafuge 2.0 R

centrifuge. Supernatant was removed and kept frozen at -20°C .

Samples of the supernatant, were analysed for amino acid content according to the Picotag method of Waters (Milford MA, USA) immediately after thawing.

- 5 In the amino acid analysis Trp and Cys values were omitted And Asp and Asn values were summed as one value. According to the data obtained, in the resulting hydrolysate the ratio between alanine and leucine (21.3 : 11.7) was 1: 0.5. Commercially available yeast hydrolysates typically exhibit alanine versus leucine ratio's of 1: 0.3 .

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- In a second experiment a yeast extract was prepared that was enriched in free glutamate. To achieve this, use was made of an endoprotease exhibiting a preference for cleaving at the C-terminal end of glutamate residues (encoded by gene nr 43 in Table 1) and a carboxypeptidase (encoded by gene nr 51 in Table 1) capable of removing these glutamate residues thus exposed. The endoprotease encoded by gene 15 nr 43 and the carboxypeptidase encoded by gene 51 (see Table 1) were overexpressed in *A.niger* according to methods described earlier. Purification of these enzymes was carried out according to procedures as described in Example 4.

- 20 The essential role of free glutamate in a number of aroma forming processes is well documented and MSG, the sodium salt of glutamic acid, is recognized as the single most important taste enhancing component.

- In this Example the pH of the 200 ml heat shocked yeast suspension is adjusted to 8.0, then the purified enzyme product encoded by gene 43 is added and the mixture was 25 incubated for 4 hours at 50 degrees C. Then the pH was lowered to 5.0 and the suspension was centrifuged. To 100milliliters of supernatant the purified gene product of gene 51 is added. Incubation with this carboxypeptidase took place for 30 minutes at 50 degrees C with continuous pH adjustments. After stopping the enzyme incubation by a heat treatment of 5 minutes by 95 degrees C, the material was again centrifuged 30 (see above) and a sample was obtained for amino acid analysis.

According to the amino acid data obtained (see above), in the resulting hydrolysate the ratio between alanine and glutamate (30.0 : 48.7) was 1: 1.6. Commercially available yeast hydrolysates typically exhibit alanine versus glutamate ratio's of 1: 1.

35

Example 10**Flavour evaluation of yeast hydrolysates enriched in specific amino acids.**

To prove that a protein hydrolysate enriched in specific amino acids according to the invention can generate specific aroma's, a number of experiments were carried out

5 with the yeast hydrolysates described in an earlier Example. To that end larger portions of these hydrolysates were prepared and lyophilised. The performance of the resulting powders were compared with the performance of a commercially available yeast extract (Gistex LS, obtainable from DSM Food Specialties, Delft, The Netherlands) in a standardised mixture under several reaction conditions. The standardised mixture

10 consisted of one of the hydrolysates, base mixture and water.

The base mixture contained 22 grams of Maxarome Plus Powder (a specialised yeast extract with a high content of natural nucleotides, also obtainable from DSM Food Specialties), 29.2 grams of glucose, 9 grams of REFEL-F fat (hydrogenated soy oil, obtainable from Barentz, Hoofddorp, The Netherlands) and 0.2 grams of calcium

15 stearyl lactylate (emulsifier, obtainable from Abitec, Northampton, UK) thoroughly mixed in a mortar.

All standardised mixtures contained 5 grams of yeast hydrolysate powder (i.e. either the leucine or the glutamate enriched material or the commercial yeast extract) , 3 grams of the base mixture and 3 grams of water. After thorough mixing, these three slurries

20 were subjected to different heating regimes i.e. either 65 minutes at 90-95 degrees C in a reaction vial (liquid reaction) or dried at 20 millibar at 120 degrees C in a vacuum oven (vacuum roast reaction) or heated in an open reaction vial at 120 degrees C for 10 minutes after the dissipation of all water (roast reaction).

After the heat treatment all three products had assumed colours ranging from dark

25 brown to almost black. In case of the vacuum roast reaction only the light coloured top layers were used. Taste evaluation of the heated products was carried out by grinding the blackened cakes into fine powders and dissolving these powders to a concentration of 2% (w/w) in water containing 0.6% (w/w) NaCl. The observations of the taste panel are specified in Table 3.

Table 3

	Reference	Leucine	Glutamate
Liquid	Bouillon, slightly roast	Cold tea, slightly flowery, yeasty	More bouillon, meaty, yeasty
Vacuum roast	Burnt, fried potatoes	Astringent, beans, yeasty	Burnt, bouillon, yeasty
Roast	Dark roast, bouillon, umami	Less roast, flowery, umami	Roast, more bouillon, more umami

5 Example 11

Non-allergenic whey protein hydrolysates formed with tripeptidylpeptidases.

The dipeptidylpeptidases encoded by the genes 19 and 55 as well as the tripeptidylpeptidases encoded by the genes 4, 9, 10, 12, 26, 35, 46, and 50 (see Table 1) may be overproduced as described and may be purified according to the methods provided in Example 4. After purification the pH optimum and the temperature stability of each individual enzyme may be established by any of the methods available and known by the skilled person. Furthermore, the specificity of each individual enzyme may be determined using the methods outlined in Example 1. The selectivity exhibited by tripeptidylpeptidases is illustrated in the following experiment.

15 The enzyme encoded by gene 12 was overproduced in an *Aspergillus niger* host cell and purified by procedures described in Example 4. The enzyme thus obtained was incubated at pH 5 and 50 degrees C with different synthetic chromogenic substrates i.e. Ala-Ala-Phe-pNA and Ala-Phe-pNA (both from Bachem, Switzerland). The incubation with the Ala-Ala-Phe-pNA substrate led to a significant increase of the absorbance at 410 nm whereas the incubation with Ala-Phe-pNA did not. This observation clearly demonstrates that tripeptidylpeptidases cleave off tripeptides and do not exhibit aminopeptidase activity that can lead to an undesirable increase of free amino acids.

Moreover, the enzyme encoded by gene 12 shows favourable enzyme stability characteristics as shown in the following experiment. Four samples of the enzyme were incubated at pH 5 for one hour at 0, 40, 50 and 60 degrees C respectively. Then each enzyme sample was incubated with the above mentioned Ala-Ala-Phe-pNA substrate in a citrate buffer at pH5 and the residual activity in each individual sample was

determined by measuring the increase in absorbance at 410 nm. With the 0 degrees C sample showing 100% activity, the 40 degrees sample showed 96% residual activity, the 50 degrees sample 92% residual activity and the 60 degrees sample 88% residual activity.

- 5 In a typical process aimed at producing a hydrolysate with a high proportion of tripeptides, whey protein (WPC 75) may be dissolved/suspended in a concentration of 100 grams of protein/liter, in an aqueous medium having a pH of 8.5. The first enzyme incubation is with the broad spectrum endoprotease subtilisin (Delvolase®, 560 000 DU per gram from DSM). After a predigestion of the whey with this enzyme in a
- 10 concentration of 0.5% enzyme concentrate per gram of protein for 2 hours at 60 degrees C, the mixture is heat-treated to inactivate the endoprotease used. Then the temperature is adjusted to 50 degrees C and the tripeptidylpeptidase is added and the whole mixture is incubated until the desired level of tripeptides is reached. Further processing steps of the hydrolysate thus obtained depend on the specific application
- 15 but may incorporate microfiltration or centrifugation followed by evaporation and spray drying.

CLAIMS

1. An isolated polynucleotide hybridisable to a polynucleotide according to a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or
5 a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114
2. An isolated polynucleotide according to claim 1 hybridisable under high stringency conditions to a polynucleotide according to a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the
10 group consisting of SEQ ID NO: 58 to SEQ ID NO: 114
3. An isolated polynucleotide according to claims 1 or 2 obtainable from a filamentous fungus.
4. An isolated polynucleotide according to claim 3 obtainable from *A. niger*.
5. An isolated polynucleotide encoding a polypeptide comprising an amino acid
15 sequence according to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 or functional equivalents thereof.
6. An isolated polynucleotide encoding at least one functional domain of a polypeptide according to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 or functional equivalents thereof.
- 20 7. An isolated polynucleotide comprising a nucleotide sequence according to a sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114 or functional equivalents thereof
8. An isolated polynucleotide according to a sequence selected from the group
25 consisting of SEQ ID NO: 1 to SEQ ID NO: 57 or a sequence selected from the group consisting of SEQ ID NO: 58 to SEQ ID NO: 114.
9. A vector comprising a polynucleotide sequence according to claims 1 to 8.
10. A vector according to claim 9 wherein said polynucleotide sequence according to claims 1 to 8 is operatively linked with regulatory sequences suitable for expression
30 of said polynucleotide sequence in a suitable host cell.

11. A vector according to claim 10 wherein said suitable host cell is a filamentous fungus
12. A method for manufacturing a polynucleotide according to claims 1 – 8 or a vector according to claims 9 to 11 comprising the steps of culturing a host cell transformed with said polynucleotide or said vector and isolating said polynucleotide or said vector from said host cell.
13. An isolated polypeptide according to a sequence selected from the group consisting of SEQ ID NO: 115 to SEQ ID NO: 171 or functional equivalents thereof.
14. An isolated polypeptide according to claim 13 obtainable from *Aspergillus niger*
15. An isolated polypeptide obtainable by expressing a polynucleotide according to claims 1 to 8 or a vector according to claims 9 to 11 in an appropriate host cell, e.g. *Aspergillus niger*.
16. Recombinant protease comprising a functional domain of a protease polypeptide.
17. A method for manufacturing a polypeptide according to claims 13 to 16 comprising the steps of transforming a suitable host cell with an isolated polynucleotide according to claims 1 to 8 or a vector according to claims 9 to 11, culturing said cell under conditions allowing expression of said polynucleotide and optionally purifying the encoded polypeptide from said cell or culture medium.
18. A recombinant host cell comprising a polynucleotide according to claims 1 to 8 or a vector according to claims 9 to 11.
19. A recombinant host cell expressing a polypeptide according to claims 13 to 16.
20. A recombinant host cell comprising a polynucleotide encoding a functionally inactivated protease polypeptide.
21. A recombinant host cell wherein a polynucleotide encoding a protease polypeptide has at least partially been deleted.
22. A recombinant host cell according to claims 18 to 21 wherein said host cell is from an *Aspergillus* species, e.g. *A. niger*.
23. A recombinant host cell functionally deficient in a protease obtainable by a method

comprising said steps of:

- a. In vitro mutagenesis of a polynucleotide according to claims 1 to 11,
 - b. Transformation of a host cell comprising an endogenous gene comprising a polynucleotide sequence hybridisable to said mutagenised polynucleotide obtained in step a),
 - c. Selecting and isolating recombinant host cells in which said endogenous gene is replaced by a mutagenised polynucleotide obtained in step a).
24. Purified antibodies reactive with a polypeptide according to claims 13 to 16.
25. Fusion protein comprising a polypeptide sequence according to claims 13 to 16.
26. Method for diagnosing whether an organism is infected with *Aspergillus* comprising said steps of:
- a. Isolating a biological sample from said organism suspected to be infected with *Aspergillus*,
 - b. Isolating nucleic acid from that sample,
 - c. Determining whether said isolated nucleic acid comprises polynucleotides hybridisable to a polynucleotide according to claims 1 to 8.
27. Method according to claim 26 wherein step c) additionally comprises amplification of said isolated nucleic acid, preferably by polymerase chain reaction.
28. Method for diagnosing whether a certain organism is infected with *Aspergillus* comprising said steps of:
- a. Isolating a biological sample from said organism suspected to be infected with *Aspergillus*,
 - b. reacting said biological sample with an antibody according to claim 24
 - c. determining whether immunocomplexes are formed.

SEQUENCE LISTING

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<120> Novel genes encoding novel proteolytic enzymes.

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<212> DNA

<213> *Aspergillus niger*

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<212> DNA
<213> Aspergillus niger

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<211> 2590

<212> DNA

<213> *Aspergillus niger*

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<210> 10

<211> 3080

<212> DNA

<213> *Aspergillus niger*

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 <211> 1890
 <212> DNA
 <213> *Aspergillus niger*

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<210> 12
<211> 3080
<212> DNA
<213> Aspergillus niger

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<210> 13
<211> 3598
<212> DNA
<213> Aspergillus niger

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<211> 2847

<212> DNA

<213> *Aspergillus niger*

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<213> *Aspergillus niger*

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<211> 2660

<212> DNA

<213> *Aspergillus niger*

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<213> *Aspergillus niger*

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<212> DNA

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<211> 2165

<212> DNA

<213> *Aspergillus niger*

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 <213> *Aspergillus niger*

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<210> 27

<211> 2660

<212> DNA

<213> *Aspergillus niger*

<400> 27

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<210> 28
<211> 1540
<212> DNA
<213> Aspergillus niger

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tgcataaagg gtgcagaaac acgaacagtg gcgtggtagc acacactctc tgcagtactc 240
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<210> 29
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 <212> DNA
 <213> *Aspergillus niger*

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<210> 30
 <211> 2380
 <212> DNA
 <213> *Aspergillus niger*

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<210> 31
<211> 2441
<212> DNA
<213> Aspergillus niger

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 <212> DNA
 <213> *Aspergillus niger*

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<211> 2287
<212> DNA
<213> Aspergillus niger

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<210> 38

<211> 1950

<212> DNA

<213> *Aspergillus niger*

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<212> DNA
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<211> 3570
 <212> DNA
 <213> *Aspergillus niger*

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<210> 43

<211> 1750

<212> DNA

<213> *Aspergillus niger*

<400> 43

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<210> 45
 <211> 3080
 <212> DNA
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<210> 46

<211> 3290

<212> DNA

<213> *Aspergillus niger*

<400> 46

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<213> *Aspergillus niger*

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<211> 2730

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<213> *Aspergillus niger*

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<213> Aspergillus niger

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<211> 2660

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<210> 57
 <211> 3916
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 <213> *Aspergillus niger*

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3916

<210> 58

<211> 1443

<212> DNA

<213> *Aspergillus niger*

<400> 58

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 <211> 3300
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<210> 60

<211> 2181

<212> DNA

<213> *Aspergillus niger*

<400> 60

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<210> 61
<211> 1695
<212> DNA
<213> Aspergillus niger

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<400> 61
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<210> 62

<211> 1581

<212> DNA

<213> *Aspergillus niger*

<400> 62

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<210> 63
<211> 3471
<212> DNA
<213> Aspergillus niger

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<210> 64

<211> 1611

<212> DNA

<213> *Aspergillus niger*

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<210> 65

<211> 840
 <212> DNA
 <213> *Aspergillus niger*

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<210> 66
 <211> 1722
 <212> DNA
 <213> *Aspergillus niger*

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<210> 67

<211> 1758

<212> DNA

<213> *Aspergillus niger*

<400> 67

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<210> 68

<211> 798

<212> DNA

<213> *Aspergillus niger*

<400> 68

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<210> 69

<211> 1743

<212> DNA

<213> *Aspergillus niger*

<400> 69

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taa 1743

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<210> 70
<211> 1896
<212> DNA
<213> Aspergillus niger

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cagcttccac tgactattga caatgtttct actgaagctg cagaggggcg gaacgagtac	540
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<210> 71
 <211> 1185
 <212> DNA

<213> *Aspergillus niger*

<400> 71

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gacaccggat ctgcagatct ctgggtcttc tcggacgagc tcccttcttc ggagcagacc      360
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<210> 72

<211> 1197

<212> DNA

<213> *Aspergillus niger*

<400> 72

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gcccattgtc gcgctctggg ccagaagtac atgggtatcc gcccgccat ccacaaagag      180
ctggtcgagg agaaccctat caatgacatg agccgtcatg atgttctggg ggacaacttc      240

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ctgaacgcac agtactttctc tgagatcgag ctgggtactc cccccagaa gttcaagggt	300
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tgctacctcc acaacaagta tgattcgtct gcctccagta cgtatcacia gaatggcagt	420
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<210> 73

<211> 1182

<212> DNA

<213> *Aspergillus niger*

<400> 73

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gcagagaaga tgtccagcac attcgcccaa ggcgaagggg acggattgct cggcttagca	540

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<210> 74

<211> 849

<212> DNA

<213> *Aspergillus niger*

<400> 74

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tacgtttaa 849

<210> 75

<211> 822

<212> DNA

<213> *Aspergillus niger*

<400> 75

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<210> 76

<211> 1629

<212> DNA

<213> *Aspergillus niger*

<400> 76

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tcagcgatgg ccactgcgtt ggagacggaa tatgccacca tccctattga ccataacaac	180
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<210> 77
<211> 1176
<212> DNA
<213> Aspergillus niger

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gagaagttct tgatcgagtt ggctccttat cagacgagat gggttaccga ggaagaaaag 180

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<210> 78
<211> 1329
<212> DNA
<213> Aspergillus niger

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<400> 78
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ggcatgatat ccgccccctt gcaccaaagt gtccaaaatg ttagcgcata tacacatgct 180
gccaactata gcaagttctc gtacgacaaa gtccatccca tcgagccagg gtctgttacc 240
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gccgatgtca acggcaccaa ttacctcga tacctgcagg aagacaccac cctggataca 360
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aatgggaagg agaataattac tatcttggag ctgttcacgc atacaagcgg ttctgcttct 540
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atcttgacgc aaaaaattat caataccccc ggcagcacat acctctactt agatctcaac 660
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ctgttgtaa 1329

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<210> 79

<211> 1839

<212> DNA

<213> *Aspergillus niger*

<400> 79

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atgcatgccg gccatattga ggtggatcca cagaacaatg gaaatctttt cttctggcac 180
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gaacgcgatg atatctacat tgccggcgag tcttacgccg gtcagcatat tccatacatc 540
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ctaaaaggcc tattgattgg caatgggttg atttctccta atgaacagta catgtcctac 660
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caggcctccc gagccggggg cagtcataat ctatcctag 1839

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<210> 80
<211> 1596
<212> DNA
<213> Aspergillus niger

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<400> 80
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taccgtgctg cgaccagagc ccagtcgagc aacacttccg actaccgatt cttcaataat 180
aggaccaagc cccacttggt ggaaagctta cccgatgtgc acttcgatgt tggggagatg 240

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ggctgttcct ccgaacaggg attctttcag gaaaatggca ggttcacatg gcagcctggg 420
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gaccaaccag tcggaaccgg attttccggt ggaaatgtta cagccaccaa cgaagaagag 540
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gagacatatc agtcggggca taagcaggct caggatcagg gccgtgtctc gtatcgccat 1560
ctgcagtggc tgttggggca agttgagatt ctttag 1596

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<210> 81
<211> 1596
<212> DNA
<213> Aspergillus niger

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<400> 81
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gagaccttcc agagcggaca catgcagccc caattccaac ccagagtgtc ataccgtcac 1560
cttgagtggc tgcttgcccg gcgggatacc ctgtaa 1596

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<210> 82
<211> 1479
<212> DNA
<213> Aspergillus niger
<400> 82

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gacaaagtct acgccaacct cctcaggcc ctcgtcgaca gcggtgccat taagtctgct      600
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acgagtactt cctcggaggg cgctcgggcg caagctacga gcaaccgat gaacctgctc     1440
ccaggacttg cgggtatcgg cctacttctc gctctgtaa                                1479

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<210> 83

<211> 1836

<212> DNA

<213> *Aspergillus niger*

<400> 83

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tacgccaaca atcctcaagg caacgaggtc attcgcttgc aaatcgccct tcagcagcat	180
gatgtcgctg gtttcgaaca agccgtgatg gatatgtcca cccccggaca cgccgactat	240
ggaaagcatt tccgcacca cgatgagatg aagcgcatgt tgctccccag cgagactgcc	300
gtcgactcag tccgcgactg gctggaatcc gccggtgtcc acaatatcca ggtcgacgcc	360
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aagtggatg tcagcgacgc caagcatatt cgtcgtctgc gcaccctgca atactccatc	480
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 aaaggggtgg cgttgggtga ggaggggtgt aattaa 1836

<210> 84
 <211> 1437
 <212> DNA
 <213> *Aspergillus niger*

<400> 84
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 gatttcgaca ttggcgagtc ctatgcgggc tacctacca acacgccttc tggaatctcg 180
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<210> 85
 <211> 633
 <212> DNA
 <213> *Aspergillus niger*

<400> 85
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 aacggcggat actacgacta cgcgggctca ttccagatgt tcaatatcga tgctgcgtat 240
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<210> 86
 <211> 1827
 <212> DNA
 <213> *Aspergillus niger*

<400> 86
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<210> 87

<211> 1251

<212> DNA

<213> *Aspergillus niger*

<400> 87

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<210> 88

<211> 1368

<212> DNA

<213> *Aspergillus niger*

<400> 88

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<210> 89

<211> 2376

<212> DNA

<213> *Aspergillus niger*

<400> 89

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<211> 1446
<212> DNA
<213> *Aspergillus niger*

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<211> 960
<212> DNA
<213> *Aspergillus niger*

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<210> 92
<211> 1920
<212> DNA
<213> *Aspergillus niger*

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<210> 93
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<210> 94
 <211> 1245
 <212> DNA
 <213> *Aspergillus niger*

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<210> 95

<211> 1443

<212> DNA

<213> *Aspergillus niger*

<400> 95

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<210> 96

<211> 1401

<212> DNA

<213> *Aspergillus niger*

<400> 96

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<210> 97

<211> 1632

<212> DNA

<213> *Aspergillus niger*

<400> 97

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<210> 98

<211> 2535

<212> DNA

<213> *Aspergillus niger*

<400> 98

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<210> 99
<211> 450
<212> DNA
<213> Aspergillus niger

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<210> 100
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<212> DNA
<213> Aspergillus niger

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<210> 101

<211> 933

<212> DNA

<213> *Aspergillus niger*

<400> 101

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<210> 102

<211> 2046

<212> DNA

<213> *Aspergillus niger*

<400> 102

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<210> 103
 <211> 1875
 <212> DNA
 <213> *Aspergillus niger*

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<210> 104

<211> 1665

<212> DNA

<213> *Aspergillus niger*

<400> 104

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<210> 105
<211> 1737
<212> DNA
<213> Aspergillus niger

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<210> 106

<211> 1371

<212> DNA

<213> *Aspergillus niger*

<400> 106

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<210> 107

<211> 1995

<212> DNA

<213> *Aspergillus niger*

<400> 107

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<210> 108
 <211> 1563
 <212> DNA
 <213> *Aspergillus niger*

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<210> 109
 <211> 1656
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 <211> 1872
 <212> DNA
 <213> *Aspergillus niger*

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 <211> 1320
 <212> DNA
 <213> *Aspergillus niger*

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 tcccaacctt tgaccatcat tgggtgcgcac caagattcgg ccaattatct ttttcccctg 720
 ctgcccgcgc ctggcgctga cgatgactgt tccggcactg tcagtatcct cgaggccttc 780
 cgcgttcttg cgagagaatgg ctacacgccc aaggacgggc ctgttgaatt ccattgggtat 840
 gcggctgaag aggccgggct actgggcagc caagccatcg cgcggtacaa gaaggagcag 900
 ggcgctaaaa ttgatgccat gatggagttt gatatgacgg cttttattgc ccgtaacgcc 960
 accgagacca tcgggtttgt tgcaacccaa gccgatgcag cgctcacaaa ctggggccctc 1020
 aacctcagtc gagaatacat ctccattccg gcggaagtct atgaacttgg cccaacgct 1080
 ggatccgact acatgtcata cactaagctc aactaccccg ctgcctttgc atccgaaggc 1140
 aaccgcctcg ctgggggctc tttcccgggt gaaatggacc cctacgtaca cggcatacag 1200
 gataggatgg acgttgacga tgaaacgggc gtcttctcta tcgaacacat ggctcggttc 1260
 tccgagttgg ctatcgcat tgttgctcag caggctgggt gggataatac atggcggtag 1320

<210> 112

<211> 1581

<212> DNA

<213> *Aspergillus niger*

<400> 112

atgcgttctt tctccgttgt cgctgccgcg tcaactggcg tctcttgggc gtctctggcc 60
 caggctgctc gccccgtct tgtgcccaag cctatctctc ggccagcttc gagtaagtcg 120
 gctgcgacta cgggtgaggc ttattttgag cagctgctgg accatcacia cccggagaag 180
 ggaacgtttt cccagcggta ctggtggagt actgaatact ggggtggacc tgggtcaccg 240
 gtggtcctct ttaaccctgg agaggtctct gccgatggct atgaggggta tctcaccaac 300
 gatactctca ctggtgtcta tgcgcaggag atccagggtg ccgtcattct cattgaacac 360
 cgctactggg gcgactcttc gccttatgag gtgctcaatg ccgaaacact tcagtatctc 420
 acactggatc agtccattct ggacatgacc tacttcgccg agacggtaaa gctgcagttc 480
 gataatagca gccgcagcaa tgcgcagaat gctccctggg tcatggctcg tggctcatac 540
 agcgggtgct tgacggcttg gaccgagtct atcgcgcctg gaacgttctg ggcttaccat 600
 gccaccagtg cgctgtgga ggctatctat gacttttggc aatacttcta cccattcag 660

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caaggtatgg cacagaactg cagcaaggat gtgtctctgg tagccgagta tgtcgacaaa 720
attgggaaga atggaactgc caaggaacag caggagctca aagaattggt tggctctggga 780
gctgttgagc attacgatga ctttgccgct gtcttgccca acggaccgta cctctggcaa 840
gacaacgact ttgtcacagg atactcttcc ttcttccagt tctgtgatgc tgtcgagggg 900
gtcgaagccg gcgcggcagt gacccccggc cccgagggcg tcggacttga aaaggccctg 960
gccaaactacg caaactgggt caattcaacc atactcccta actactgcgc aagctacggc 1020
tactggaccg acgaatggag cgtcgcctgt ttcgacagct ataatgcctc gagccccatc 1080
ttcaccgaca cctccgtggg taacctgtc gaccgccaat gggaatgggt cctctgcaac 1140
gagcctttct tctggtggca ggacggtgcc cccgagggaa cctccactat tgtgccccgg 1200
ctcgtcagcg cctcctactg gcaacgccaa tgcccgtct acttcccoga agttaacggc 1260
tacacgtacg gcagcgcgaa gggtaaaaac tccgctacgg tgaacagctg gacgggtgga 1320
tgggatatga cccgcaacac gacgcggttg atctggacga acgggcaata tgaccctgg 1380
cgcgactccg gtgtgtcgag cactttccgg cccggtggtc cgctggttag cacggcgaac 1440
gaaccctgac agattattcc gggcggttc cattgctcgg acttgatat ggaggattac 1500
tatgcgaatg aggggtgtgag gaagggtggt gataatgagg tgaagcagat taaggagtgg 1560
gtggaggagt attatgcttg a 1581

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<210> 113
<211> 1275
<212> DNA
<213> Aspergillus niger

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<400> 113
atgcagctcc tccagtcctt cattgttgcc gtttgcttca gctacggcgt cctctcctta 60
ccccatggcc cgtcaaacca gcacaaagca cgttccttca aggttgaacg ggtccgtcgt 120
ggaaccgggtg ctctgcatgg gcccgctgct ctccgcaaag cataccggaa gtacggaata 180
gctcccagca gtttcaacat cgatctggca gactttaaac ccattacgac aaccatgct 240
gctgctggga gcgagattgc agagcctgat cagactggcg ctgtcagtgc tacttccgtc 300
gagaacgatg ccgagttcgt ttcgcctgtt cttattggcg gccagaagat cgtcatgaca 360
tttgacactg gttcttctga cttttgggtg ttcgatacga atctcaatga aaccttgacg 420
ggacacacgg agtacaaccc ttcgaactcc tcgaccttca agaagatgga cggatacacc 480
ttcgatgtct cgtatggtga cgactcgtac gcctctggcc ccgtcggaac ggataccgtc 540

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aacattggcg gcgccattgt caaggagcaa gccttcggtg tccccgacca ggtatcccag 600
 tcgttcacgc aggacacgaa ctccaacggc ctggtcgggt tgggcttttc ctccatcaac 660
 accatcaaac cggaggcgca agacacgttc ttcgccaatg tcgcaccaag tctggacgag 720
 cccgtcatga ccgcctcgct caaggctgac ggagtgggag agtacgagtt cggcacgac 780
 gacaaagaca agtaccaggg caacattgcc aacatcagcg tggactcatc gaacggatac 840
 tggcagttct ccactcccaa gtactccgtg gcagacggag agctgaagga cattggaagc 900
 ttgaacacct cgatcgcgga caccggtacc tcccttatgc tgctggatga agacgtgggt 960
 actgcctact atgcgcaagt tcccaactcg gtctacgtga gcagtgccgg tggttacatc 1020
 tacccttgca acaccactct tcccagcttc tcgcttgctc tcggcgagtc gagcctggcc 1080
 acgatccccg gtaacctgat caatttctcc aaggttggca ccaacaccac caccggacag 1140
 gccttgctgt ttggcggcat tcaatccaac ggaaacacct cgctgcagat tctgggcat 1200
 attttctga aggccttttt cgttgtcttc gacatgcgag gccctcgct tgggtgtgac 1260
 tctcccaaga actag 1275

<210> 114
 <211> 1647
 <212> DNA
 <213> *Aspergillus niger*

<400> 114
 atgcgcattg actccgcggc gctacatctg gtcccagtc tcttgggcca ggtcgggtgct 60
 ttacaattac ccttgggtcca agactccaat tcacagtggc agaaaccaa tgcaggtgat 120
 aaacccttaa ttagctctcc gttgcttcaa gagcaggtea aggcggagaa tctgttggac 180
 agggcccggc agctttacaa gattgcggag ctgggagaag acgagtataa cccccact 240
 cgcgtcattg gcagtaaagg tcaccttggc acgctcgact acatatactc cacccttacc 300
 gacctcgggtg attattatac tgtcgtcaat cagtccctcc ctgccgtgag cggtaatgtc 360
 ttcgagtctc gccttgctct tggtcacgat gttcccaagt cagctacacc aatgggtctc 420
 actcccccaa cgaggaataa ggagccggta tatggctccc tggttgctgt atccaacctc 480
 ggggtgtgagg cctcggacta ctctccaac ttgaaaggcg ccgttgcatc tatcagtcgg 540
 ggaagctgtc cgttcgggac caagtctcaa ttagctggta aagcgggagc tgttgctgcc 600
 gtcattctaca acaacgagcg gggtgacctc agcggaaactc taggaaaccc aacccccgat 660
 catgttgcta cctttggtat ctacagcag gatgctgccc cagtcctgga gaagttgaat 720

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aaaggcgaga aggtggacgc tatcgctac gttgatgca tagtagagac catccacacc 780
accaatatca tcgcgagac cacggatggt gacccgaaca attgtgtaat gctgggtggc 840
cacagtgaca gcggtggccga gggcccgggt atcaatgacg acgggtccgg tactctgacc 900
cttttgagc ttgccacatt gctcaccag ttccgtgtca acaactgcgt gcgatttgct 960
tggtgggccc cggaggagga aggccttctc ggatctgact attacgtgtc cgttctcaca 1020
ccggaagaga accgcaagat ccgcttgctc atggactacg acatgctcgg ctgcccgaac 1080
tttgcgtagc aagtttaca tgccactaat gctgtgaacc ccgagggatc tgaggagctt 1140
cgtgatctgt acaccgactt ttacgaagat catgggttca actacacgta cattccgttt 1200
gacggacgca ggcactatga tgccttcatt cggcatggta tcccgggtgg tggcattgcc 1260
acgggagcag agggatatcaa gactgtcgag gaagcggaca tgtttggtgg ggttgctggc 1320
caatggtatg acccgtgtta ccatcagatc tgcgatacgg tggccaatgt gaacttgact 1380
gcgtgggagt ggaacaccaa gctcgttgcc cactccattg cgacttacgc caagtccttt 1440
gacggattcc cggaacggtc cgatgaaccc atcagccctg ctgcttttga ggaaccgaag 1500
taccatggcc acgcgttgca attacttcgc ggtaatacta cagggacca gagcgtcctg 1560
tggggagccc aaatccagaa tggaacagct gcacgggtgc ttaatctatt gtccatacga 1620
cgcagaggca ctttcagtct aagctaa 1647

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<210> 115
<211> 480
<212> PRT
<213> Aspergillus niger

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<400> 115

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Met His Leu Pro Gln Arg Leu Val Thr Ala Ala Cys Leu Cys Ala Ser
1           5           10           15

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Ala Thr Ala Phe Ile Pro Tyr Thr Ile Lys Leu Asp Thr Ser Asp Asp
20           25           30

```

```

Ile Ser Ala Arg Asp Ser Leu Ala Arg Arg Phe Leu Pro Val Pro Lys
35           40           45

```

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Pro Ser Asp Ala Leu Ala Asp Asp Ser Thr Ser Ser Ala Ser Asp Glu
50           55           60

```

Ser Leu Ser Leu Asn Ile Lys Arg Ile Pro Val Arg Arg Asp Asn Asp
 65 70 75 80

Phe Lys Ile Val Val Ala Glu Thr Pro Ser Trp Ser Asn Thr Ala Ala
 85 90 95

Leu Asp Gln Asp Gly Ser Asp Ile Ser Tyr Ile Ser Val Val Asn Ile
 100 105 110

Gly Ser Asp Glu Lys Ser Met Tyr Met Leu Leu Asp Thr Gly Gly Ser
 115 120 125

Asp Thr Trp Val Phe Gly Ser Asn Cys Thr Ser Thr Pro Cys Thr Met
 130 135 140

His Asn Thr Phe Gly Ser Asp Asp Ser Ser Thr Leu Glu Met Thr Ser
 145 150 155 160

Glu Glu Trp Ser Val Gly Tyr Gly Thr Gly Ser Val Ser Gly Leu Leu
 165 170 175

Gly Lys Asp Lys Leu Thr Ile Ala Asn Val Thr Val Arg Met Thr Phe
 180 185 190

Gly Leu Ala Ser Asn Ala Ser Asp Asn Phe Glu Ser Tyr Pro Met Asp
 195 200 205

Gly Ile Leu Gly Leu Gly Arg Thr Asn Asp Ser Ser Tyr Asp Asn Pro
 210 215 220

Thr Phe Met Asp Ala Val Ala Glu Ser Asn Val Phe Lys Ser Asn Ile
 225 230 235 240

Val Gly Phe Ala Leu Ser Arg Ser Pro Ala Lys Asp Gly Thr Val Ser
 245 250 255

Phe Gly Thr Thr Asp Lys Asp Lys Tyr Thr Gly Asp Ile Thr Tyr Thr
 260 265 270

Asp Thr Val Gly Ser Asp Ser Tyr Trp Arg Ile Pro Val Asp Asp Val
 275 280 285

Tyr Val Gly Gly Thr Ser Cys Asp Phe Ser Asn Lys Ser Ala Ile Ile

290 295 300
 Asp Thr Gly Thr Ser Tyr Ala Met Leu Pro Ser Ser Asp Ser Lys Thr
 305 310 315 320
 Leu His Ser Leu Ile Pro Gly Ala Lys Ser Ser Gly Ser Tyr His Ile
 325 330 335
 Ile Pro Cys Asn Thr Thr Thr Lys Leu Gln Val Ala Phe Ser Gly Val
 340 345 350
 Asn Tyr Thr Ile Ser Pro Lys Asp Tyr Val Gly Ala Thr Ser Gly Ser
 355 360 365
 Gly Cys Val Ser Asn Ile Ile Ser Tyr Asp Leu Phe Gly Asp Asp Ile
 370 375 380
 Trp Leu Leu Gly Asp Thr Phe Leu Lys Asn Val Tyr Ala Val Phe Asp
 385 390 395 400
 Tyr Asp Glu Leu Arg Val Gly Phe Ala Glu Arg Ser Ser Asn Thr Thr
 405 410 415
 Ser Ala Ser Asn Ser Thr Ser Ser Gly Thr Ser Ser Thr Ser Gly Ser
 420 425 430
 Thr Thr Thr Gly Ser Ser Thr Thr Thr Thr Ser Ser Ala Ser Ser Ser
 435 440 445
 Ser Ser Ser Asp Ala Glu Ser Gly Ser Ser Met Thr Ile Pro Ala Pro
 450 455 460
 Gln Tyr Phe Phe Ser Ala Leu Ala Ile Ala Ser Phe Met Leu Trp Leu
 465 470 475 480

 <210> 116
 <211> 1099
 <212> PRT
 <213> Aspergillus niger

 <400> 116
 Met Leu Arg Gly Leu Arg Asp Val Val Leu Leu Gln Phe Ala Ile Pro
 1 5 10 15

Leu Phe Leu Leu Leu His Phe Arg Leu Ser Leu Arg Gly Val Ile Thr
 20 25 30
 Gly Phe Gly Ser Lys Ser His Phe Gln Arg Pro Leu Ser Lys Met Ser
 35 40 45
 Ser Thr Gln Lys Ser His Phe Lys Leu Leu Gln Lys Phe Lys Pro Glu
 50 55 60
 Tyr Ser Pro Ser Glu Phe Ala Gln Tyr Glu Ser Glu Arg Thr Gly Met
 65 70 75 80
 Arg Val Val Val Ile Asp Gln Lys Gly Pro Lys Val Thr Gly Tyr Phe
 85 90 95
 Val Leu Ala Thr Glu Ile Leu Asp Asp Ser Gly Ala Pro His Thr Leu
 100 105 110
 Glu His Leu Cys Phe Met Gly Ser Arg Asn Tyr Arg Tyr Lys Gly Phe
 115 120 125
 Leu Asp Lys Leu Ala Thr Arg Val Tyr Ser Ser Thr Asn Ala Trp Thr
 130 135 140
 Ala Thr Asp His Thr Ala Tyr Thr Leu Asp Thr Ala Gly Trp Glu Gly
 145 150 155 160
 Phe Ala Gln Ile Leu Pro Val Tyr Leu Glu His Val Ile Ala Pro Thr
 165 170 175
 Leu Thr Asp Glu Gly Cys Tyr Thr Glu Val His His Ile Asp Gly Ala
 180 185 190
 Gly Asp Asp Ala Gly Val Val Tyr Ser Glu Met Gln Gly Val Gln Asn
 195 200 205
 Asn Ser Ala Glu Leu Ile Asp Leu Thr Ala Arg Arg Leu Thr Tyr Pro
 210 215 220
 His Gly Val Gly Phe Arg Tyr Glu Thr Gly Gly Met Met Glu Gln Leu
 225 230 235 240

Arg Val Leu Thr Ala Asp Arg Ile Arg Ala Phe His Arg Glu Met Tyr
 245 250 255

Gln Pro Lys Asn Leu Cys Leu Ile Ile Thr Gly Glu Val Asp His Gln
 260 265 270

Asn Met Leu Glu Thr Leu Asp Lys Phe Glu Asp Thr Ile Leu Asp Val
 275 280 285

Ile Pro Ser Pro Asp Ser Pro Phe Lys Arg Pro Trp Val Asp Ser Lys
 290 295 300

Gln Ala Pro Pro Leu Glu Lys Ser Ile Val Gln Thr Val Glu Phe Pro
 305 310 315 320

Glu Glu Asp Glu Ser Phe Gly Glu Ile Glu Ile Arg Phe Leu Gly Pro
 325 330 335

Asp Cys Thr Asp Pro Val Gln Thr Gly Ala Val Asn Val Ala Leu Leu
 340 345 350

Tyr Leu Ala Gly Ser Ser Ala Ser Leu Leu Asp Asn Ile Leu Val Glu
 355 360 365

Lys Glu Gln Leu Ala Ser Ala Val Tyr Tyr Ala Thr Glu Asp His Pro
 370 375 380

Ser Ile Glu Ile Arg Phe Thr Leu Thr Ser Val Glu Thr Glu Lys Leu
 385 390 395 400

Ala Lys Val Glu Gln Arg Phe Phe Glu Val Leu Lys Asp Ala Met Glu
 405 410 415

Lys Asp Leu Asp Met Arg Tyr Ile Lys Glu Cys Ile Asp Arg Gln Arg
 420 425 430

Arg Thr Trp Lys Phe Ser Thr Glu Ser Ser Ala Ser Ser Phe Ala Glu
 435 440 445

Tyr Val Ile Ser Asp Phe Leu Phe Gly Lys Arg Asp Gly Ser Thr Met
 450 455 460

Leu Asp Val Ala Thr Leu Gln Glu Tyr Asp Val Leu Glu Lys Trp Ser

465		470		475		480
Glu Glu Gln Trp	Arg Ser Phe Ile Lys Thr Trp Ile Ser Asp Ala Asn					
	485		490		495	
His Val Thr Ile	Leu Gly Val Pro Ser Val Lys Met Ser Asp Thr Leu					
	500		505		510	
Lys Lys Glu Glu Glu Ala Arg Val Ala Glu Gln Lys Lys Arg Leu Gly						
	515		520		525	
Asp Glu Gly Leu Lys Lys Leu Ala Asp Lys Leu Glu Lys Ala Lys Ala						
	530		535		540	
Glu Asn Asp Lys Glu Ile Pro Lys Glu Met Leu Glu Arg Phe Gln Ile						
545		550		555		560
Pro Gly Ile Glu Ser Ile His Phe Val Asp Thr Thr Thr Ala Arg Ser						
	565		570		575	
Gly Ala Ala Leu Asp Ala Gly Arg Pro Ser His Lys Ala Gln Lys Leu						
	580		585		590	
Val Asp Ala Asp Gly Ser Asp Leu Pro Leu Phe Ile His Phe Glu His						
	595		600		605	
Ile Pro Ser Ser Phe Val Gln Leu Ser Leu Leu Ile Ser Ala Gln Ala						
610		615		620		
Val Pro Val Gln Leu Arg Pro Leu Leu Ser Val Tyr Thr Glu Ala Phe						
625		630		635		640
Phe Asn Leu Pro Val Asn Arg Asn Gly Glu Thr Ile Asn Phe Glu Gln						
	645		650		655	
Val Val Val Glu Leu Glu Arg Asp Thr Val Gly Tyr Ser Met Glu Gly						
	660		665		670	
Ala Arg Ser Leu Gly Asn Ser Glu Met Leu Arg Ile Ser Phe Gln Val						
	675		680		685	
Glu Leu Glu Lys Tyr His Thr Ala Ile Ala Trp Ile Gln Glu Leu Ser						
690		695		700		

Trp Asn Ser Ile Phe Asp Val Glu Arg Leu Arg Ala Ile Thr Ser Arg
705 710 715 720

Leu Leu Ser Asp Val Pro Asp Ser Lys Arg Ser Gly Asp Asp Met Leu
725 730 735

Ala Ala Val His Val Met Val His Tyr Ala Ala Glu Ser Ile Val Arg
740 745 750

Ala Arg Ser Thr Leu Val Lys Ala Arg Tyr Leu Lys Arg Ile Lys Lys
755 760 765

Gln Leu Ala Glu Glu Pro Lys Ser Val Val Ala Arg Met Glu Glu Ile
770 775 780

Arg Asp Ala Leu Phe Arg Phe Glu Asn Met Arg Val Leu Val Ile Ala
785 790 795 800

Asp Leu Glu Lys Leu Gln Asn Pro Val Ser Ala Trp Lys Pro Phe Ala
805 810 815

Glu Arg Leu Gly Ala Gly Ala Pro Leu Gln Pro Ile Thr Thr Arg Arg
820 825 830

Pro Leu Leu Ser Glu Ala Gly Gln Lys Leu Gly Gly Lys Ser Tyr Val
835 840 845

Val Pro Met Pro Thr Ile Asp Ser Ser Phe Ala Tyr Ala Thr Ala Arg
850 855 860

Gly Leu Asp Ser Tyr Asp Asp Pro Arg Leu Pro Ala Leu Met Val Ala
865 870 875 880

Ile Ala Tyr Met Asn Ala Val Glu Gly Pro Leu Trp Val Ala Val Arg
885 890 895

Gly Lys Gly Leu Ala Tyr Gly Thr Asn Phe Ala Tyr Asn Ile Asp Thr
900 905 910

Gly Phe Val Asn Phe Asp Val Tyr Arg Ser Pro Asn Ala His Lys Ala
915 920 925

Phe Asp Ser Ser Lys Gln Ile Val Glu Asp His Leu Ser Gly Ala Met
 930 935 940

Pro Phe Asp Pro Leu Met Leu Glu Gly Ser Ile Ser Ser Ile Val Val
 945 950 955 960

Ser Phe Ala Asn Glu Gln Ser Thr Ile Gly Ser Ala Ala Ser Gly Ser
 965 970 975

Phe Ile Arg Gln Val Ile Arg Arg Leu Pro Ser Asp Tyr Lys Glu Arg
 980 985 990

Val Leu Lys Gln Val Arg Ala Thr Ser Val Asp Asp Val Lys Gly Ala
 995 1000 1005

Leu Lys Asp Ile Ile Leu Pro Leu Phe Asn Pro Ser Thr Ala Asn
 1010 1015 1020

Ile Val Val Thr Cys Ala Thr Val Leu Glu Glu Thr Ile Lys Glu
 1025 1030 1035

Gly Leu Gln Ala Ser Gly Phe Thr Pro Ala Val Gln Pro Leu Lys
 1040 1045 1050

Glu Phe Glu Asp Asp Tyr Gly Leu Lys Val Gly Asp Asp Glu Asp
 1055 1060 1065

Glu Glu Ser Asp Asp Asp Asp Asp Glu Tyr Glu Thr Gly Ser Glu
 1070 1075 1080

Asp Glu Asp Asp Ser Asp Glu Asp Met Glu Asp Asp Glu Asp Asp
 1085 1090 1095

Glu

<210> 117
 <211> 726
 <212> PRT
 <213> Aspergillus niger

<400> 117

Met Gly Ala Leu Gln Trp Leu Ser Ile Thr Ala Ala Ala Ala Ser Ala

[illegible]

Ser Pro Asp Gly Lys Trp Val Thr Phe Lys Ser Lys Ala Pro Glu Leu
245 250 255

Pro Leu Ala Asn Asn Thr Ala Ala Tyr Val Tyr Leu Val Pro His Asp
260 265 270

Gly Ser Ala Thr Ala Phe Ala Val Asn Gly Pro Asp Ser Pro Ala Thr
275 280 285

Pro Glu Gly Val Glu Gly Glu Ser Asn Asn Pro Val Phe Ser Pro Asp
290 295 300

Ser Asp Lys Ile Ala Tyr Phe Gln Met Ala Thr Asn Thr Tyr Glu Ser
305 310 315 320

Asp Arg Asn Val Leu Tyr Val Tyr Ser Ile Ala Asp Asp Thr Ile Thr
325 330 335

Pro Leu Ala Lys Asp Trp Asp Arg Ser Pro Ser Ser Val Thr Trp Val
340 345 350

Asp Gly Asp Asn Leu Val Val Ala Ser Gln Asp Leu Gly Arg Thr Arg
355 360 365

Leu Phe Ala Ile Pro Gly Asp Ala Gly Asp Asp Phe Lys Pro Thr Asn
370 375 380

Phe Thr Asp Gly Gly Ser Val Ser Ala Gln Tyr Val Leu Ser Asn Ser
385 390 395 400

Thr Leu Leu Val Thr Ser Ser Ala Phe Trp Thr Ser Trp Ser Val Tyr
405 410 415

Thr Ala Ser Pro Asp Glu Gly Val Ile Asn Thr Leu Ala Ser Ala Asn
420 425 430

Glu Ile Asp Pro Glu Leu Ser Gly Leu Ser Ser Ser Asp Phe Glu Glu
435 440 445

Phe Tyr Phe Asp Gly Asn Trp Thr Thr Leu Gln Gly Trp Ile Thr Tyr
450 455 460

Pro Gln Asp Phe Asp Ser Ser Lys Lys Tyr Pro Leu Ala Phe Leu Ile
465 470 475 480

His Gly Gly Pro Glu Asp Ala Trp Ala Asp Glu Trp Asn Leu Lys Trp
485 490 495

His Ser Lys Val Phe Ala Asp Gln Gly Tyr Val Val Val Gln Pro Asn
500 505 510

Pro Thr Gly Ser Thr Gly Phe Gly Gln Gln Leu Thr Asp Ala Ile Gln
515 520 525

Leu Asn Trp Thr Gly Ala Ala Tyr Asp Asp Leu Thr Lys Ala Trp Gln
530 535 540

Tyr Val His Asp Thr Tyr Asp Phe Ile Asp Thr Asp Asn Gly Val Ala
545 550 555 560

Ala Gly Pro Ser Phe Gly Ala Phe Met Ile Thr Trp Ile Gln Gly Asp
565 570 575

Asp Phe Gly Arg Lys Phe Lys Ala Leu Val Ser His Asp Gly Pro Phe
580 585 590

Ile Gly Asp Ala Trp Val Glu Thr Asp Glu Leu Trp Phe Val Glu His
595 600 605

Glu Phe Asn Gly Thr Phe Trp Gln Ala Arg Asp Ala Phe His Asn Thr
610 615 620

Asp Pro Ser Gly Pro Ser Arg Val Leu Ala Tyr Ser Thr Pro Gln Leu
625 630 635 640

Val Ile His Ser Asp Lys Asp Tyr Arg Ile Pro Val Ala Asn Gly Ile
645 650 655

Gly Leu Phe Asn Thr Leu Gln Glu Arg Gly Val Pro Ser Arg Phe Leu
660 665 670

Asn Phe Pro Asp Glu Asp His Trp Val Thr Gly Gln Glu Asn Ser Leu
675 680 685

Val Trp Tyr Gln Gln Val Leu Gly Trp Ile Asn Arg Tyr Ser Gly Val
 690 695 700

Gly Gly Ser Asn Pro Asp Ala Ile Ala Leu Glu Asp Thr Val Asn Pro
 705 710 715 720

Val Val Asp Leu Asn Pro
 725

<210> 118
 <211> 564
 <212> PRT
 <213> *Aspergillus niger*

<400> 118

Met Thr Arg Gln Thr Ser Leu Val Pro Arg Leu Leu Thr Leu Ala Ser
 1 5 10 15

Leu Ala Ala Leu Ser Gln Ala Glu Leu Gly Lys Ile Gln Trp Lys Gly
 20 25 30

Ser Cys Asn Leu Thr Thr Tyr Pro Ala Leu Ile Cys Gly Thr Leu Asp
 35 40 45

Val Pro Tyr Asp Tyr Thr Glu Ser Asn Ser Ser Lys Thr Leu Thr Leu
 50 55 60

Asp Ile Ala Lys Trp Pro Ala Thr Lys Lys Pro Val Ser Glu Pro Ile
 65 70 75 80

Ile Phe Asn Phe Gly Gly Pro Gly Val Asn Ser Phe Glu Gly Leu Gly
 85 90 95

Leu Tyr Gly Glu Glu Phe Gln Ala Ile Leu Gly Gly His Asn Asp Leu
 100 105 110

Ile Ala Phe Asn Asn Arg Gly Val Gly Asn Thr Ile Pro Phe Ser Cys
 115 120 125

Tyr Ser Asp Asp Ala Thr Arg Glu Leu Val Ala Leu Gln Ala Pro Asn
 130 135 140

Asp Gly Arg Ala Ser Ser Thr Ala Leu Gly Glu Ile Trp Ala Gln Asn
 145 150 155 160

Ala Asn Ile Ala Gln Ala Cys Tyr Ala Thr Asn Asn Gln Thr Gly Ser
 165 170 175

Leu Ile Gly Thr Ser Phe Ala Ala Arg Asp Ile Met Gln Val Ala Asp
 180 185 190

Ala Leu Ser Gly Lys Asp Ser Leu Val Asn Tyr Trp Gly Phe Ser Tyr
 195 200 205

Gly Thr Thr Ile Gly Ala Val Leu Ala Ala Met Phe Pro Asp Arg Met
 210 215 220

Gly Asn Val Ala Leu Asp Gly Val Asp Asn Pro Arg Glu Ala Leu Tyr
 225 230 235 240

Gly Tyr Asn Ala Gln Ala Val Val Asp Val Asp Lys Val Phe Glu Gly
 245 250 255

Phe Cys Thr Gly Cys Met Ala Ala Pro Asp Leu Cys Pro Ile Ala Lys
 260 265 270

Glu Tyr Thr Ser Ala Ala Asn Leu Glu Ala Ala Ile Tyr Leu Met Leu
 275 280 285

Glu Asn Leu Lys Tyr Asn Pro Ile Ala Ile Pro Glu Thr Gly Gly Ile
 290 295 300

Val Thr Trp Ser Asp Val Lys Ser Thr Ile Phe Glu Ala Met Tyr Leu
 305 310 315 320

Pro Ser Ser Trp Pro Leu Thr Ser Glu Leu Leu Tyr Tyr Val Gln Thr
 325 330 335

Arg Asn Thr Thr Ile Leu Gly Asn Ser Glu Val Tyr Asp Thr Ile Lys
 340 345 350

Ser Tyr Gly Gln Ser Ala Ser Leu Thr Ser Ala Ser Asp Glu Val Gly
 355 360 365

Thr Ala Ile Thr Cys Ser Asp Lys His Arg Ser Ala Thr Ile Lys Glu
 370 375 380

Val Leu Pro Tyr Val Lys Ala Arg Gln Ala Leu Thr Lys Ile Gly Ser
385 390 395 400

Asp Gly Ser Asp Gly Asp Met Arg Cys Ala Gln Trp Asn Pro Lys Met
405 410 415

Phe Ala Lys Glu Arg Tyr Ser Gly Asp Phe Glu Val Lys Thr Ala Asn
420 425 430

Pro Val Leu Ile Leu Ser Asn Thr Tyr Asp Pro Ala Thr Pro Leu Pro
435 440 445

Ala Ala Lys Asn Leu Thr Glu Thr Phe Glu Gly Ser Val Leu Leu Glu
450 455 460

Gln Asn Gly Tyr Gly His Thr Thr Leu Ser Met Pro Ser Leu Cys Thr
465 470 475 480

Ala Lys Ala Val Arg Ala Tyr Phe Thr Asn Gly Thr Leu Pro Ala Asp
485 490 495

Gly Thr Ile Cys Gln Val Asp Val Pro Leu Phe Thr Asn Leu Thr Tyr
500 505 510

Lys Asp Val Trp Pro Lys Ser Phe Gln Arg Ser Val Glu Ser Arg Asp
515 520 525

Asp Ala Thr Ile Leu Lys Ala Leu Met Ser Val Arg Asp Lys Met Ser
530 535 540

Arg Arg Arg Met Cys Ile Tyr Leu Tyr Thr Asn Ser Ala Ser Trp Arg
545 550 555 560

Pro Glu Leu Pro

<210> 119

<211> 526

<212> PRT

<213> Aspergillus niger

<400> 119

Met Tyr Tyr Ser Leu Trp Val Ala Ala Leu Val Ala Ala Leu Pro Val

1	5	10	15
Ser Arg Ala Gln Phe Val Ala Pro Pro Thr Asp Leu Ile Pro Thr Lys	20	25	30
Gly Tyr Leu Asp Ile Pro Val Arg Tyr Lys Gln Val Pro Thr Gly Ile	35	40	45
Cys Glu Thr Asp Pro Ser Val Lys Ser Phe Ser Gly Tyr Val Asp Val	50	55	60
Ala Glu His Glu His Ile Phe Phe Trp Phe Phe Glu Ala Arg Asn Gln	65	70	75
Asp Pro Thr Glu Ala Pro Leu Thr Val Trp Ile Asn Gly Gly Met Ser	85	90	95
Asp Pro Gly Pro Gly Ser Ser Ser Met Ile Gly Leu Phe Gln Glu His	100	105	110
Gly Pro Cys Gly Ile Asp Ala Asn Gly Ser Val Tyr Asn Asn Pro Tyr	115	120	125
Ser Trp Asn Asn Ala Ser Asn Met Leu Tyr Ile Asp Gln Pro Val Gln	130	135	140
Thr Gly Phe Ser Tyr Ser Ile Pro Val Pro Gly Tyr Val Asp Ser Ser	145	150	155
Thr Asp Asn Gly Phe Met Gly Ala Phe Pro Gln Tyr Ser Arg Glu Thr	165	170	175
Phe His Phe Thr Thr Glu Ser Tyr Gly Gly His Tyr Gly Pro Val Phe	180	185	190
Asn Glu Tyr Ile Glu Glu Gln Asn Ala His Leu Gln Pro Gly Ala Lys	195	200	205
Lys Ile Gln Leu Gly Ser Val Met Ile Gly Asn Gly Trp Tyr Asp Pro	210	215	220
Ile Ile Gln Tyr Gln Ala Tyr Tyr Asn Phe Thr Val Tyr Pro Gly Asn	225	230	235
			240

Thr Tyr Asp Tyr Leu Pro Phe Asn Lys Ser Ile Ser Ser Leu Met Tyr
 245 250 255

Asn Asn Leu Tyr Gly Pro Gly Asn Cys Leu Asp Gln Leu Tyr Asp Cys
 260 265 270

Ala Ala Arg Gly Ile Asp Glu Ile Cys Ser Thr Ala Asp Asp Phe Cys
 275 280 285

Ala Asn Glu Val Glu Asn Val Tyr Asp Ile Tyr Ser Gly Arg Asp Glu
 290 295 300

Tyr Asp Phe Arg Glu Leu Thr Pro Asp Pro Phe Pro Tyr Glu Phe Tyr
 305 310 315 320

Val Asp Tyr Leu Asn Lys Ala Ser Val Gln Ala Ala Ile Gly Ala Tyr
 325 330 335

Ile Asn Tyr Thr Glu Ser Asn Asn Ala Val Gly Leu Ala Phe Ser Ser
 340 345 350

Thr Gly Asp Asp Gly Arg Leu Met Asn Thr Ile Gln Asp Val Gly Lys
 355 360 365

Leu Leu Lys Gln Gly Val Thr Val Val Met Tyr Ala Gly Asp Ala Asp
 370 375 380

Tyr Asn Cys Asn Trp Leu Gly Gly Glu Ala Val Ser Leu Gln Val Lys
 385 390 395 400

Ala Ala Asn Phe Ser Ser Ala Gly Tyr Thr Asn Ile Val Thr Ser Asp
 405 410 415

Gly Val Thr His Gly Gln Val Arg Gln Ala Gly Gln Phe Ala Phe Val
 420 425 430

Arg Val Tyr Glu Ser Gly His Glu Val Pro Phe Tyr Gln Pro Leu Leu
 435 440 445

Ala Leu Glu Met Phe Glu Arg Val Ile Gly Gly Lys Asp Val Ala Thr
 450 455 460

Gly Lys Ile Pro Ile Ser Ser Ser Leu Gln Thr Val Gly Thr Pro Lys
465 470 475 480

Ser Tyr Tyr Arg Glu Gly Asn Ser Thr Ile Gln Trp Glu Val Leu Asp
485 490 495

Ser Leu Ala Thr Tyr Asn Thr Thr Thr Asn Ala Pro Asn Pro Val Ser
500 505 510

Arg Arg Leu Lys Arg Met Gly Pro Ala Leu Arg Phe Gln Met
515 520 525

<210> 120
<211> 1156
<212> PRT
<213> Aspergillus niger

<400> 120

Met Ser Cys Val Trp Leu His Ile His Lys Arg Ser Leu Leu Ser Val
1 5 10 15

Ala Thr Asn Asn Ser Val Ala Arg Ala Ala Ala Ser Thr Ser Ala Ala
20 25 30

Pro Pro Pro Pro Ser Ser Pro Pro Pro Gly Ser Asn Thr Tyr Ser Pro
35 40 45

Leu Tyr Arg Pro Ile Thr Asn Pro Ile Gly Phe Thr Leu Ser Pro Ala
50 55 60

Arg Ser Leu Val Ser Arg Asn Pro Lys Phe Pro Ala Tyr Arg Arg Ser
65 70 75 80

Ser Arg His Phe Ser Leu Cys Pro Ala Ala Ala Thr Pro Gly Val Thr
85 90 95

Thr Ser Ile Cys Pro Gly Gln Ala Pro Val Arg Ser Leu Ser Ser Leu
100 105 110

Ile Ile His Ser Thr Arg Pro Arg Ala Ile Arg Ile Arg Thr Asp Gln
115 120 125

Met Asp Leu Asn Gly Asp Ala Gly Ala Lys Arg Lys Arg Ser Ser Ile

130					135					140					
Thr	Thr	Pro	Ala	Glu	Arg	Pro	Val	Lys	His	Leu	Arg	Pro	Glu	Ser	Ser
145					150					155					160
Ala	Leu	Thr	Pro	Gly	Asp	Ser	Thr	Pro	Ala	Asn	Gly	Thr	Val	Tyr	Asp
				165					170					175	
Val	Glu	Asp	Asp	Glu	Asp	Ala	Ser	Arg	Leu	Leu	Pro	Val	Gly	Pro	Ala
			180					185					190		
Gln	Ala	Asp	Ser	Pro	Glu	Trp	Gln	Ala	Thr	Ile	Glu	Glu	Val	Val	Lys
		195					200					205			
Ser	Val	Val	Ser	Ile	His	Phe	Cys	Gln	Thr	Cys	Ser	Phe	Asp	Thr	Glu
	210					215					220				
Leu	Ser	Met	Ser	Ser	Gln	Ala	Thr	Gly	Phe	Val	Val	Asp	Ala	Glu	Asn
225					230					235					240
Gly	Tyr	Ile	Leu	Thr	Asn	Arg	His	Val	Val	Cys	Pro	Gly	Pro	Phe	Trp
				245					250					255	
Gly	Tyr	Cys	Ile	Phe	Asp	Asn	His	Glu	Glu	Cys	Asp	Val	Arg	Pro	Val
			260					265					270		
Tyr	Arg	Asp	Pro	Val	His	Asp	Phe	Gly	Ile	Leu	Lys	Phe	Asp	Pro	Lys
		275					280					285			
Ala	Ile	Arg	Tyr	Met	Lys	Leu	Arg	Glu	Leu	Lys	Leu	Gln	Pro	Asp	Ala
	290					295					300				
Ala	Lys	Val	Gly	Ser	Glu	Ile	Arg	Val	Val	Gly	Asn	Asp	Ala	Gly	Glu
305					310					315					320
Lys	Leu	Ser	Ile	Leu	Ser	Gly	Val	Ile	Ser	Arg	Leu	Asp	Arg	Asn	Ala
				325					330					335	
Pro	Glu	Tyr	Gly	Asp	Gly	Tyr	Ser	Asp	Phe	Asn	Thr	Asn	Tyr	Ile	Gln
			340					345					350		
Ala	Ala	Ala	Ala	Ala	Ser	Gly	Gly	Ser	Ser	Gly	Ser	Pro	Val	Val	Asn
			355				360					365			

Ile Asp Gly His Ala Ile Ala Leu Gln Ala Gly Gly Arg Ala Asp Gly
 370 375 380

Ala Ala Thr Asp Tyr Phe Leu Pro Leu Asp Arg Pro Leu Arg Ala Leu
 385 390 395 400

Glu Cys Ile Arg Arg Gly Glu Pro Val Thr Arg Gly Thr Ile Gln Thr
 405 410 415

Gln Trp Ile Leu Lys Pro Phe Asp Glu Cys Arg Arg Leu Gly Leu Thr
 420 425 430

Pro Glu Trp Glu Ala Thr Val Arg Lys Ala Ala Pro Thr Glu Thr Ser
 435 440 445

Met Leu Val Ala Glu Ile Ile Leu Pro Glu Gly Pro Ala Asp Gly Lys
 450 455 460

Leu Glu Glu Gly Asp Val Leu Leu Gln Val Asn Gly Val Leu Leu Thr
 465 470 475 480

Gln Phe Ile Arg Leu Asp Asp Ile Leu Asp Ser Ser Val Gly Gln Thr
 485 490 495

Val Arg Leu Leu Val Gln Arg Gly Gly Gln Asn Val Glu Ile Glu Cys
 500 505 510

Gln Val Gly Asp Leu His Ala Ile Thr Pro Asp Arg Phe Val Thr Val
 515 520 525

Ala Gly Gly Thr Phe His Asn Leu Ser Tyr Gln Gln Ser Arg Leu Tyr
 530 535 540

Ala Ile Ala Thr Arg Gly Val Tyr Val Cys Glu Ala Ala Gly Ser Phe
 545 550 555 560

Lys Leu Glu Asn Thr Leu Ser Gly Trp Ile Ile Asp Ser Val Asp Lys
 565 570 575

Arg Pro Thr Arg Asn Leu Asp Glu Phe Val Glu Val Met Arg Thr Ile
 580 585 590

Pro Asp Arg Ser Arg Val-Val Ile Ser Tyr Arg His Ile Arg Asp Leu
 595 600 605

His Thr Arg Gly Thr Ser Ile Val Tyr Ile Asp Arg His Trp His Pro
 610 615 620

Lys Met Arg Leu Ala Val Arg Asn Asp Asp Thr Gly Leu Trp Asp Phe
 625 630 635 640

Ser Asp Leu Ala Asp Pro Ile Pro Ala Leu Pro Pro Val Pro Arg Lys
 645 650 655

Ala Asp Phe Ile Gln Leu Asp Gly Val Ser Gln Pro Ala Ala Ala Asp
 660 665 670

Ile Val Arg Ser Phe Val Arg Val Ser Cys Thr Met Pro Leu Lys Leu
 675 680 685

Asp Gly Tyr Pro Gln Ala Lys Lys Thr Gly Phe Gly Leu Val Val Asp
 690 695 700

Ala Glu Lys Gly Leu Val Val Val Ser Arg Ala Ile Val Pro Tyr Asp
 705 710 715 720

Leu Cys Asp Ile Asn Val Thr Val Ala Asp Ser Ile Ile Val Asn Ala
 725 730 735

Lys Val Val Phe Leu His Pro Leu Gln Asn Tyr Ser Ile Ile Gln Tyr
 740 745 750

Asp Pro Ser Leu Val Gln Ala Pro Val Gln Ser Ala Lys Leu Ala Thr
 755 760 765

Asp Tyr Ile Lys Gln Gly Gln Asp Thr Ile Phe Val Gly Phe Asn Gln
 770 775 780

Asn Phe Arg Ile Val Val Ala Lys Thr Ala Val Thr Asp Ile Thr Thr
 785 790 795 800

Val Ser Ile Pro Ala Asn Ala Ser Ala Pro Arg Tyr Arg Ala Ile Asn
 805 810 815

Leu Asp Ala Ile Thr Val Asp Thr Gly Leu Ser Gly Gln Cys Ser Asn
 820 825 830

Gly Val Leu Ile Gly Glu Asp Gly Val Val Gln Ala Leu Trp Leu Asn
 835 840 845

Tyr Leu Gly Glu Arg Thr Ser Asn Ser His Lys Asp Val Glu Tyr His
 850 855 860

Leu Gly Phe Ala Thr Pro Ser Leu Leu Pro Val Leu Ser Lys Val Gln
 865 870 875 880

Gln Gly Glu Met Pro Glu Leu Arg Ile Leu Asn Met Glu Ser Tyr Val
 885 890 895

Val Gln Met Ser Gln Ala Arg Ile Met Gly Val Ser Glu Glu Trp Ile
 900 905 910

Glu Lys Val Thr Gln Ala Asn Pro Ser Arg His Gln Leu Phe Met Val
 915 920 925

Arg Lys Val Asp Cys Pro Pro Pro Gly Phe Asn Ser Ala Ala Asp Thr
 930 935 940

Phe Glu Glu Gly Asp Ile Ile Leu Thr Leu Asp Gly Gln Leu Ile Thr
 945 950 955 960

Arg Val Ser Glu Leu Asp Ile Met Tyr Glu Lys Asp Thr Leu Glu Ala
 965 970 975

Leu Ile Val Arg Asn Gly Gln Glu Met Arg Ile Gln Val Pro Thr Val
 980 985 990

Pro Thr Glu Asp Leu Glu Thr Asp Arg Ala Val Val Phe Cys Gly Ala
 995 1000 1005

Val Leu Gln Lys Pro His His Ala Val Arg Gln Gln Ile Ser Lys
 1010 1015 1020

Leu His Ser Glu Val Tyr Val Ser Ala Arg Ser Arg Gly Ser Pro
 1025 1030 1035

Ser Tyr Gln Tyr Gly Leu Ala Pro Thr Asn Phe Ile Thr Ala Val

1040 1045 1050
 Asn Gly Val Pro Thr Pro Asn Leu Asp Arg Phe Ser Glu Glu Val
 1055 1060 1065
 Ser Lys Ile Pro Asp Asn Thr Tyr Phe Arg Leu Arg Ala Val Thr
 1070 1075 1080
 Phe Asp Asn Val Pro Trp Val Val Thr Val Lys Lys Asn Asp His
 1085 1090 1095
 Tyr Phe Pro Met Ser Glu Tyr Ile Lys Asp Gln Ser Gln Pro Ser
 1100 1105 1110
 Gly Trp Arg Thr Val Ser His Asp Lys Asp Lys Tyr Lys Asp Gly
 1115 1120 1125
 Ile Ala Pro Asp Ala Ala Asn Leu Asn Pro Asp Ala Met Asp Glu
 1130 1135 1140
 Gly Phe Asp Gly Val Ser Asp Ile Glu Pro Asp Leu Glu
 1145 1150 1155

 <210> 121
 <211> 536
 <212> PRT
 <213> Aspergillus niger

 <400> 121

 Met Arg Val Leu Pro Ala Ala Met Leu Val Gly Ala Ala Thr Ala Ala
 1 5 10 15

 Val Pro Pro Phe Gln Gln Val Leu Gly Gly Asn Gly Ala Lys His Gly
 20 25 30

 Ala Asp His Ala Ala Glu Val Pro Ala Asp His Ser Ala Asp Gly Phe
 35 40 45

 Ser Lys Pro Leu His Ala Phe Gln Glu Glu Leu Lys Ser Leu Ser Asp
 50 55 60

 Glu Ala Arg Lys Leu Trp Asp Glu Val Ala Ser Phe Phe Pro Glu Ser
 65 70 75 80

Met Asp Gln Asn Pro Leu Phe Ser Leu Pro Lys Lys His Asn Arg Arg
 85 90 95

Pro Asp Ser His Trp Asp His Ile Val Asp Gly Lys Leu Glu Ala Tyr
 100 105 110

Asp Leu Arg Val Lys Lys Thr Asp Pro Gly Ser Leu Gly Ile Asp Pro
 115 120 125

Gly Val Lys Gln Tyr Thr Gly Tyr Leu Asp Asp Asn Glu Asn Asp Lys
 130 135 140

His Leu Phe Tyr Trp Phe Phe Glu Ser Arg Asn Asp Pro Glu Asn Asp
 145 150 155 160

Pro Val Val Leu Trp Leu Asn Gly Gly Pro Gly Cys Ser Ser Leu Thr
 165 170 175

Gly Leu Phe Met Glu Leu Gly Pro Ser Ser Ile Asn Lys Lys Ile Gln
 180 185 190

Pro Val Tyr Asn Asp Tyr Ala Trp Asn Ser Asn Ala Ser Val Ile Phe
 195 200 205

Leu Asp Gln Pro Val Asn Val Gly Tyr Ser Tyr Ser Asn Ser Ala Val
 210 215 220

Ser Asp Thr Val Ala Ala Gly Lys Asp Val Tyr Ala Leu Leu Thr Leu
 225 230 235 240

Phe Phe Lys Gln Phe Pro Glu Tyr Ala Lys Gln Asp Phe His Ile Ala
 245 250 255

Gly Glu Ser Tyr Ala Gly His Tyr Ile Pro Val Phe Ala Ser Glu Ile
 260 265 270

Leu Ser His Lys Lys Arg Asn Ile Asn Leu Gln Ser Val Leu Ile Gly
 275 280 285

Asn Gly Leu Thr Asp Gly Tyr Thr Gln Tyr Glu Tyr Tyr Arg Pro Met
 290 295 300

Ala Cys Gly Asp Gly Gly Tyr Pro Ala Val Leu Asp Glu Ser Ser Cys
 305 310 315 320

Gln Ser Met Asp Asn Ala Leu Pro Arg Cys Gln Ser Met Ile Glu Ser
 325 330 335

Cys Tyr Ser Ser Glu Ser Ala Trp Val Cys Val Pro Ala Ser Ile Tyr
 340 345 350

Cys Asn Asn Ala Leu Leu Ala Pro Tyr Gln Arg Thr Gly Gln Asn Val
 355 360 365

Tyr Asp Val Arg Gly Lys Cys Glu Asp Ser Ser Asn Leu Cys Tyr Ser
 370 375 380

Ala Met Gly Tyr Val Ser Asp Tyr Leu Asn Lys Pro Glu Val Ile Glu
 385 390 395 400

Ala Val Gly Ala Glu Val Asn Gly Tyr Asp Ser Cys Asn Phe Asp Ile
 405 410 415

Asn Arg Asn Phe Leu Phe His Gly Asp Trp Met Lys Pro Tyr His Arg
 420 425 430

Leu Val Pro Gly Leu Leu Glu Gln Ile Pro Val Leu Ile Tyr Ala Gly
 435 440 445

Asp Ala Asp Phe Ile Cys Asn Trp Leu Gly Asn Lys Ala Trp Thr Glu
 450 455 460

Ala Leu Glu Trp Pro Gly Gln Ala Glu Tyr Ala Ser Ala Glu Leu Glu
 465 470 475 480

Asp Leu Val Ile Val Asp Asn Glu His Thr Gly Lys Lys Ile Gly Gln
 485 490 495

Val Lys Ser His Gly Asn Phe Thr Phe Met Arg Leu Tyr Gly Gly Gly
 500 505 510

His Met Val Pro Met Asp Gln Pro Glu Ser Ser Leu Glu Phe Phe Asn
 515 520 525

Arg Trp Leu Gly Gly Glu Trp Phe

530

535

<210> 122

<211> 279

<212> PRT

<213> Aspergillus niger

<400> 122

Met Lys Phe Thr Asn Tyr Leu Leu Thr Thr Ala Thr Leu Ala Ser Ser
 1 5 10 15

Val Leu Ala Ala Pro Ala Pro Arg Thr Gly Leu Glu Asp Arg Leu Arg
 20 25 30

Ala Arg Ser Leu Gln Arg Gln Ser His Pro Leu Ala Pro Ile Pro Leu
 35 40 45

Asp Thr Ser Thr Lys Glu Asn Ser Arg Leu Leu Glu Ala Asp Glu Asn
 50 55 60

Thr Thr His Val Thr Tyr Ser Ser Asn Trp Ala Gly Ala Val Arg Glu
 65 70 75 80

Gln Pro Pro Pro Gln Gly Thr Tyr Ser Ala Val Ser Ala Thr Phe Arg
 85 90 95

Val Pro Glu Pro Thr Ala Gln Gly Gly Ser Gly Thr Gln Ala Gly Ser
 100 105 110

Ala Trp Val Gly Ile Asp Gly Asp Thr Tyr Ser Asn Ala Ile Leu Gln
 115 120 125

Thr Gly Val Asp Phe Tyr Val Glu Asn Gly Gln Thr Tyr Asn Asp Ala
 130 135 140

Trp Tyr Glu Trp Tyr Pro Asp Tyr Ala Tyr Asp Phe Asp Leu Asp Val
 145 150 155 160

Ser Thr Gly Asp Thr Ile Val Ala Lys Val Glu Ala Ile Ser Pro Ser
 165 170 175

Gln Gly Val Ala Thr Ile Glu Asn Ile Ser Thr Gly Lys Lys Ala Thr
 180 185 190

Gln Thr Ile Arg Ala Pro Ala Ala Thr Ala Thr Leu Ala Gly Gln Asn
 195 200 205

Ala Asp Trp Ile Val Glu Asp Phe Gln Ser Gly Asp Ser Met Val Asp
 210 215 220

Leu Ala Gly Phe Gly Glu Ile Ser Phe Trp Gly Val Gln Ala Gln Gly
 225 230 235 240

Gly Gly Ser Thr Trp Gly Val Asp Asp Ala Thr Ile Val Glu Leu Lys
 245 250 255

Gln Gly Asn Glu Val Leu Thr Asp Val Glu Val Gln Ser Asp Ser Ala
 260 265 270

Phe Thr Val Lys Tyr Thr Ser
 275

<210> 123
 <211> 573
 <212> PRT
 <213> *Aspergillus niger*

<400> 123

Met Ile Tyr Val Asn Tyr Ile Leu Gly Leu Leu Ser Leu Leu His Thr
 1 5 10 15

Ala Val Ala Thr Ala Pro Asp Tyr Val Val Val Asp Gln Leu Asn Ser
 20 25 30

Ile Pro Asp Gly Trp Thr Lys Gly Ala Ala Pro Pro Pro Phe Thr Pro
 35 40 45

Met Lys Phe Trp Leu Ser Met His His Glu Tyr Lys Ala Asp Phe Glu
 50 55 60

Gln Lys Val Ile Asp Ile Ser Thr Pro Gly His Arg Asp Tyr Gly Arg
 65 70 75 80

His Met Lys Arg Asn Asp Val Met Ala Phe Met Arg Pro Ser Asp Gln
 85 90 95

Val Ser Lys Ile Ile Phe Ser Trp Leu Glu Ser Glu His Val Pro Pro

100	105	110
Asn Ala Ile Glu Asp Arg Gly Asp Trp Val Ala Phe Thr Val Pro Leu		
115	120	125
Ala Gln Ala Gln Ser Met Met Lys Thr Asp Phe Tyr Asn Phe His His		
130	135	140
Leu Glu Thr Asn Thr Thr Gln Ile Arg Thr Leu Lys Tyr Ser Val Pro		
145	150	155
Glu Gln Val Asp Ala His Leu Gln Met Ile Gln Pro Thr Thr Arg Phe		
165	170	175
Gly Arg Pro Lys Thr Gln Thr Ser Leu Pro Ser Leu Met Pro Val Ser		
180	185	190
Val Asn Ile Asp Glu Ile Ser Glu Asp Cys Leu Thr Gly Val Thr Pro		
195	200	205
Ile Cys Leu Arg Gln Leu Tyr Gly Leu Pro Ser Thr Lys Ala Ser Pro		
210	215	220
Asp Ser Arg Asn Val Leu Gly Ile Ser Gly Tyr Leu Asp Gln Tyr Ala		
225	230	235
Arg Tyr Ser Asp Leu Asp Glu Phe Leu Ala Val Tyr Ser Pro Asn Ser		
245	250	255
Val Asp Ala Asp Phe Ser Val Val Ser Ile Asn Gly Gly Gln Asn Pro		
260	265	270
Gln Asn Ser Gln Glu Gly Ser Thr Glu Ala Ser Leu Asp Ile Gln Tyr		
275	280	285
Ala Leu Ser Met Ala Phe Asp Ala Asn Ala Thr Phe Tyr Thr Thr Ala		
290	295	300
Gly Arg Ala Pro Ser Pro Tyr Leu Glu Gln Leu Gln Tyr Leu Val Gly		
305	310	315
Leu Pro Asp Glu Asp Leu Pro Ala Val Leu Ser Thr Ser Tyr Gly Glu		
325	330	335

Asp Glu Gln Ser Leu Pro Glu Glu Tyr Thr Glu Ala Thr Cys Asn Leu
 340 345 350

Phe Ala Gln Leu Gly Ala Arg Gly Val Ser Val Ile Phe Ser Ser Gly
 355 360 365

Asp Ser Gly Val Gly Gly Ser Cys Val Ser Asn Asp Gly Ser Gln Arg
 370 375 380

Thr Arg Phe Gln Pro Ile Phe Pro Ala Ser Cys Pro Phe Val Thr Ser
 385 390 395 400

Val Gly Gly Thr Glu Gly Val Gly Pro Glu Lys Ala Val Asp Phe Ser
 405 410 415

Ser Gly Gly Phe Ser Glu Arg Phe Ala Arg Pro Ser Tyr Gln Asn Ala
 420 425 430

Ser Val Glu Ala Tyr Leu Ala Arg Leu Gly Asp Lys Trp Asp Gly Leu
 435 440 445

Tyr Asn Pro Asp Gly Arg Gly Ile Pro Asp Val Ser Ala Gln Ala Ser
 450 455 460

Asn Tyr Val Ile Arg Asp His Gly Gln Trp Leu Gln Thr Ala Gly Thr
 465 470 475 480

Ser Ala Ala Ala Pro Val Phe Ala Ala Val Ile Ser Arg Leu Asn Ala
 485 490 495

Ala Arg Leu Glu Gln Gly Lys Pro Thr Leu Gly Phe Leu Asn Pro Trp
 500 505 510

Leu Tyr Ser Leu Asp Gln Gln Gly Phe Thr Asp Ile Val Asp Gly Gly
 515 520 525

Ser Val Gly Cys Asp Gly Ser Asn Gly Gly Ala Leu Val Pro Tyr Ala
 530 535 540

Ser Trp Asn Ala Thr Lys Gly Trp Asp Pro Val Thr Gly Leu Gly Thr
 545 550 555 560

Pro Leu Tyr Gln Thr Leu Glu Gln Leu Ala Gln Ser Ala
 565 570

<210> 124
 <211> 585
 <212> PRT
 <213> *Aspergillus niger*

<400> 124

Met Arg Ser Ser Gly Leu Tyr Thr Ala Leu Leu Cys Ser Leu Ala Ala
 1 5 10 15

Ser Thr Asn Ala Ile Val His Glu Lys Leu Ala Ala Val Pro Ser Gly
 20 25 30

Trp His His Val Glu Asp Ala Gly Ser Asp His Gln Ile Ser Leu Ser
 35 40 45

Ile Ala Leu Ala Arg Lys Asn Leu Asp Gln Leu Glu Ser Lys Leu Lys
 50 55 60

Asp Leu Ser Thr Pro Gly Glu Ser Gln Tyr Gly Gln Trp Leu Asp Gln
 65 70 75 80

Glu Asp Val Asp Thr Leu Phe Pro Val Ala Ser Asp Lys Ala Val Ile
 85 90 95

Asn Trp Leu Arg Ser Ala Asn Ile Thr His Ile Ser Arg Gln Gly Ser
 100 105 110

Leu Val Asn Phe Ala Thr Thr Val Asp Lys Val Asn Lys Leu Leu Asn
 115 120 125

Ala Thr Phe Ala Tyr Tyr Gln Ser Gly Ser Ser Gln Arg Leu Arg Thr
 130 135 140

Thr Glu Tyr Ser Ile Pro Asp Asp Leu Val Asp Ser Ile Asp Leu Ile
 145 150 155 160

Ser Pro Thr Thr Phe Phe Gly Lys Glu Lys Thr Thr Ala Gly Leu Asn
 165 170 175

Gln Arg Ala Gln Lys Ile Asp Thr His Val Ala Lys Arg Ser Asn Ser

180	185	190
Ser Ser Cys Ala Asp Val Ile Thr Leu Ser Cys Leu Lys Glu Met Tyr 195 200 205		
Asn Phe Gly Asn Tyr Thr Pro Ser Ala Ser Ser Gly Ser Lys Leu Gly 210 215 220		
Phe Gly Ser Phe Leu Asn Glu Ser Ala Ser Tyr Ser Asp Leu Ala Lys 225 230 235 240		
Phe Glu Lys Leu Phe Asn Leu Pro Ser Gln Ser Phe Ser Val Glu Leu 245 250 255		
Val Asn Gly Gly Val Asn Asp Gln Asn Gln Ser Thr Ala Ser Leu Thr 260 265 270		
Glu Ala Asp Leu Asp Val Glu Leu Leu Val Gly Val Ala His Pro Leu 275 280 285		
Pro Val Thr Glu Phe Ile Thr Ser Gly Glu Pro Ala Ala Asp Asn Glu 290 295 300		
Asn Glu Pro Tyr Leu Gln Tyr Tyr Glu Tyr Leu Leu Ser Lys Pro Asn 305 310 315 320		
Ser Ala Leu Pro Gln Val Ile Ser Asn Ser Tyr Gly Asp Asp Glu Gln 325 330 335		
Thr Val Pro Glu Tyr Tyr Ala Lys Arg Val Cys Asn Leu Ile Gly Leu 340 345 350		
Val Gly Leu Arg Gly Ile Ser Val Leu Glu Ser Ser Gly Asp Glu Gly 355 360 365		
Ile Gly Ser Gly Cys Arg Thr Thr Asp Gly Thr Asn Arg Thr Gln Phe 370 375 380		
Asn Pro Ile Phe Pro Ala Thr Cys Pro Tyr Val Thr Ala Val Gly Gly 385 390 395 400		
Thr Met Ser Tyr Ala Pro Glu Ile Ala Trp Glu Ala Ser Ser Gly Gly 405 410 415		

Phe Ser Asn Tyr Phe Glu Arg Ala Trp Phe Gln Lys Glu Ala Val Gln
 420 425 430

Asn Tyr Leu Ala His His Ile Thr Asn Glu Thr Lys Gln Tyr Tyr Ser
 435 440 445

Gln Phe Ala Asn Phe Ser Gly Arg Gly Phe Pro Asp Val Ala Ala His
 450 455 460

Ser Phe Glu Pro Ser Tyr Glu Val Ile Phe Tyr Gly Ala Arg Tyr Gly
 465 470 475 480

Ser Gly Gly Thr Ser Ala Ala Cys Pro Leu Phe Ser Ala Leu Val Gly
 485 490 495

Met Leu Asn Asp Ala Arg Leu Arg Ala Gly Lys Ser Thr Leu Gly Phe
 500 505 510

Leu Asn Pro Leu Leu Tyr Ser Lys Gly Tyr Arg Ala Leu Thr Asp Val
 515 520 525

Thr Gly Gly Gln Ser Ile Gly Cys Asn Gly Ile Asp Pro Gln Asn Asp
 530 535 540

Glu Thr Val Ala Gly Ala Gly Ile Ile Pro Trp Ala His Trp Asn Ala
 545 550 555 560

Thr Val Gly Trp Asp Pro Val Thr Gly Leu Gly Leu Pro Asp Phe Glu
 565 570 575

Lys Leu Arg Gln Leu Val Leu Ser Leu
 580 585

<210> 125

<211> 265

<212> PRT

<213> Aspergillus niger

<400> 125

Met Lys Thr Thr Ala Leu Leu Thr Ala Gly Leu Leu Ala Thr Thr Ala
 1 5 10 15

Met Ala Ala Pro Leu Thr Ala Lys Arg Gln Ala Ala Arg Ala Lys Arg
 20 25 30

Ser Thr Asn Arg Gln Ser Asn Pro Pro Phe Lys Pro Gly Thr Asn Glu
 35 40 45

Val Leu Ala Leu Asn Gly Thr Lys Asn Val Glu Tyr Ser Ser Asn Trp
 50 55 60

Ala Gly Ala Val Leu Ile Gly Thr Gly Tyr Thr Ala Val Thr Ala Glu
 65 70 75 80

Phe Val Val Pro Thr Pro Ser Val Pro Ser Gly Gly Ser Ser Arg Glu
 85 90 95

Glu Tyr Cys Ala Ser Ala Trp Val Gly Ile Asp Gly Asp Thr Cys Asp
 100 105 110

Thr Ala Ile Leu Gln Thr Gly Val Asp Phe Cys Val Gln Gly Ser Glu
 115 120 125

Val Ser Phe Asp Ala Trp Tyr Glu Trp Tyr Pro Asp Tyr Ala Tyr Asp
 130 135 140

Phe Ser Gly Ile Ser Ile Ser Ala Gly Asp Thr Ile Lys Val Thr Val
 145 150 155 160

Asp Ala Ser Ser Asp Thr Thr Gly Thr Ala Thr Ile Glu Asn Val Ser
 165 170 175

Thr Gly Thr Thr Val Thr His Ser Phe Thr Gly Gly Val Asp Gly Asp
 180 185 190

Leu Cys Glu Tyr Asn Ala Glu Trp Ile Val Glu Asp Phe Glu Glu Asp
 195 200 205

Asp Ser Leu Val Pro Phe Ala Asp Phe Gly Thr Val Thr Phe Thr Ser
 210 215 220

Cys Ser Ala Thr Lys Asp Gly Ser Ser Val Gly Pro Glu Asp Ala Thr
 225 230 235 240

Ile Ile Asp Ile Glu Gln Asn Glu Val Leu Thr Ser Val Ser Val Ser

245 250 255

Ser Ser Glu Val Val Val Lys Tyr Val
260 265

<210> 126
<211> 580
<212> PRT
<213> Aspergillus niger

<400> 126

Met Val Ala Phe Ser Arg Ile Ser Ala Gly Phe Ala Leu Ala Ala Pro
1 5 10 15

Ala Leu Ala Ser Val Val Leu Glu Thr Val Lys Ser Val Pro Ser Asp
20 25 30

Trp Lys Leu Val Glu Ala Ala Asp Thr Ser Ser Thr Ile Ser Leu Ser
35 40 45

Val Ala Leu Ala Arg Gln Asn Leu Asp Gln Leu Glu Glu Lys Leu Leu
50 55 60

Ala Val Ser Thr Pro Gly Lys Asp Thr Tyr Gly Gln Phe Leu Asp Leu
65 70 75 80

Asp Asp Ile Asn Glu Gln Phe Pro Leu Ala Asp Asp Ala Ala Val Val
85 90 95

Ala Trp Leu Lys Lys Ala Gly Val Thr Gln Ile His Lys Glu Gly Gly
100 105 110

Leu Leu Asn Phe Ala Thr Thr Val Gly Thr Ala Asn Gln Leu Leu Asn
115 120 125

Thr Thr Phe Ser Val Tyr Lys Ser Gly Ser Thr Gln Lys Leu Arg Thr
130 135 140

Thr Gln Tyr Ser Val Pro Asp Glu Leu Thr Gly Ser Ile Asp Leu Ile
145 150 155 160

Ser Pro Thr Val Phe Phe Gly Lys Ser Asn Ala Ala Arg Ser Ala Ala
165 170 175

Val Arg Ala Ser Gln Thr Thr Lys Glu Thr Ser Arg Lys Lys Ser Ser
 180 185 190

Asn Val Cys Glu Tyr Ile Thr Pro Asp Cys Leu Lys Glu Gln Tyr Ser
 195 200 205

Ile Asp Tyr Thr Pro Glu Ala Ser Ser Gly Ser Arg Val Gly Phe Gly
 210 215 220

Ser Phe Leu Asn Glu Ser Ala Leu Tyr Ser Asp Leu Asp Leu Phe Thr
 225 230 235 240

Gln Tyr Phe Asp Ile Pro Gln Gln Ser Phe Thr Val Glu Thr Ile Asn
 245 250 255

Gly Gly Ile Asn Asn Gln Glu Asn Asp Pro Asp Gly Glu Ala Asp Leu
 260 265 270

Asp Val Gln Asn Ile Val Gly Ile Ser His Pro Leu Pro Val Thr Glu
 275 280 285

Tyr Ile Thr Gly Gly Ser Pro Pro Phe Ile Pro Asp Val Glu Thr Thr
 290 295 300

Thr Asp Glu Asn Glu Pro Tyr Leu Gln Tyr Tyr Glu Tyr Leu Leu Ala
 305 310 315 320

Lys Thr Asn Asp Glu Leu Pro Leu Val Ile Ser Asn Ser Tyr Gly Asp
 325 330 335

Asp Glu Asp Thr Val Pro Ile Ala Tyr Ala Thr Arg Val Cys Asn Leu
 340 345 350

Ile Gly Leu Met Gly Thr Arg Gly Ile Ser Ile Leu Glu Ser Ser Gly
 355 360 365

Asp Ser Gly Val Gly Gly Ala Cys Met Ser Asn Asp Gly Thr Asp Lys
 370 375 380

Thr Glu Phe Thr Pro Met Phe Pro Gly Thr Cys Pro Tyr Ile Thr Ala
 385 390 395 400

Val Gly Gly Thr Gln Asp Val Pro Glu Val Ala Trp Val Asp Ser Ser
 405 410 415

Gly Gly Phe Ser Asn Tyr Phe Ser Gln Pro Ser Tyr Gln Ser Asp Gln
 420 425 430

Val Glu Thr Tyr Leu Asp Lys Tyr Ile Ser Ala Ser Thr Lys Lys Tyr
 435 440 445

Tyr Glu Gln Tyr Thr Asn Phe Ser Gly Arg Ala Phe Pro Asp Val Ser
 450 455 460

Ala Phe Ala Gly Ser Pro Tyr Tyr Glu Thr Tyr Ile Asp Gly Gln Leu
 465 470 475 480

Gly Leu Val Ala Gly Thr Ser Gly Ala Ser Pro Val Phe Ala Gly Ile
 485 490 495

Val Ala Leu Leu Asn Asp Ala Arg Leu Arg Ala Asn Lys Thr Ser Leu
 500 505 510

Gly Phe Leu Asn Pro Trp Leu Tyr Ser Ser Gly Tyr Lys Ser Leu Asn
 515 520 525

Asp Ile Thr Ser Gly Glu Ala Val Gly Cys Gln Gly Asp Val Glu Gly
 530 535 540

Ala Gly Val Ile Pro Trp Ala Ser Trp Asn Ala Thr Thr Gly Trp Asp
 545 550 555 560

Pro Ala Thr Gly Leu Gly Thr Pro Asn Phe Ala Lys Leu Lys Glu Ala
 565 570 575

Val Leu Ala Leu
 580

<210> 127

<211> 631

<212> PRT

<213> *Aspergillus niger*

<400> 127

Met His Gly Leu Arg Leu Val Cys Ser Ile Gly Thr Leu Pro Leu Val
 1 5 10 15

Ile Leu Ala Tyr Pro Ala Ala Ser Leu His Thr Thr Ser Ala Ala Val
 20 25 30

Asp Leu Asp Ser Leu Arg Leu Thr Ser Asn Ser Glu Tyr Val Asn Ser
 35 40 45

Val His Val Asp Thr Asn Arg Ser Val Ala Val Ser Ala Glu Glu His
 50 55 60

Tyr Thr Asp Thr Ala Ala Arg Leu Val Gln Asn Ile Val Pro Gly Ala
 65 70 75 80

Ser Phe Arg Leu Ile Asp Asp His Phe Val Gly Asp Asn Gly Val Ala
 85 90 95

His Val Tyr Phe Arg Gln Thr Leu His Gly Ile Asp Ile Asp Asn Ala
 100 105 110

Asp Phe Asn Val Asn Ile Gly Lys Asp Gly Leu Val Leu Ser Phe Gly
 115 120 125

His Ser Phe Phe Thr Gly Ala Leu Pro Ser Ser His Leu Asp Asn Thr
 130 135 140

Asn Val Leu Ser Pro Glu Ala Ala Leu Arg Gly Ala Arg Asp Ala Ile
 145 150 155 160

Gln Leu Pro Leu Thr Ile Asp Asn Val Ser Thr Glu Ala Ala Glu Gly
 165 170 175

Arg Asn Glu Tyr Ile Phe Arg Glu Ala Val Gly Ala Val Ser Asp Pro
 180 185 190

Lys Ala Lys Leu Val Tyr Leu Val Lys Pro Glu Gly Thr Leu Ala Leu
 195 200 205

Thr Trp Arg Ile Glu Thr Asp Met Tyr Glu His Trp Leu Leu Thr Tyr
 210 215 220

Ile Asp Ala Glu Thr Thr Thr Val His Gly Val Val Asp Tyr Val Ala
 225 230 235 240

Asp Ala Thr Tyr Gln Val Tyr Pro Trp Gly Thr Asn Asp Pro Ala Glu
 245 250 255

Gly His Arg Thr Ile Val Thr Asp Pro Trp Asp Leu Ser Ala Ser Ala
 260 265 270

Tyr Thr Trp Ile Ser Asp Gly Arg Asp Asn Tyr Thr Thr Thr Arg Gly
 275 280 285

Asn Asn Ala Ile Ala His Trp Asn Pro Thr Gly Gly Gly Ser Tyr Leu
 290 295 300

Tyr Asn Leu Arg Pro Ser Asp Pro Asn Leu Asn Phe Gln Trp Pro Tyr
 305 310 315 320

Ser Pro Asn Met Ser Pro Pro Arg Ser Tyr Ile Asn Ala Ser Ile Val
 325 330 335

Gln Leu Phe Tyr Thr Ala Asn Ala Tyr His Asp Leu Leu Tyr Thr Leu
 340 345 350

Gly Phe Thr Glu Ser Ala Gly Asn Phe Gln Trp Asn Asn Ser Ala His
 355 360 365

Gly Gly Arg Asp Lys Asp Tyr Val Ile Leu Asn Ala Gln Asp Gly Ser
 370 375 380

Gly Phe Ser Asn Ala Asn Phe Ala Thr Pro Pro Asp Gly Ile Pro Gly
 385 390 395 400

Arg Met Arg Met Tyr Ile Trp Ile Glu Ser Thr Pro Ser Arg Asp Gly
 405 410 415

Ser Phe Asp Ala Gly Ile Val Ile His Glu Tyr Thr His Gly Val Ser
 420 425 430

Asn Arg Leu Thr Gly Gly Ser His Asn Ala Gly Cys Leu Ser Ala Leu
 435 440 445

Glu Ser Gly Gly Met Gly Glu Gly Trp Gly Asp Phe Met Ala Thr Ala
 450 455 460

Ile Arg Ile Lys Pro Asn Asp Thr Arg Thr Thr Ser Tyr Thr Met Gly
465 470 475 480

Ala Trp Ala Asp Asn Asp Lys Cys Gly Val Arg Asp Tyr Pro Tyr Ser
485 490 495

Thr Ser Phe Thr Glu Asn Pro Leu Asn Tyr Thr Ser Val Asn Thr Met
500 505 510

Asn Gly Val His Ala Ile Gly Thr Val Trp Ala Thr Met Leu Tyr Glu
515 520 525

Val Leu Trp Asn Leu Ile Asp Lys Tyr Gly Lys Asn Asp Gly Ser Arg
530 535 540

Pro Val Phe Arg Asn Gly Val Pro Thr Asp Gly Lys Tyr Leu Met Met
545 550 555 560

Lys Leu Val Val Asp Gly Met Ala Leu Gln Pro Cys Asn Pro Asn Phe
565 570 575

Val Gln Ala Arg Asp Ala Ile Leu Asp Ala Asp Ile Val Leu Thr Gly
580 585 590

Gly Lys Asn Arg Cys Glu Ile Trp Arg Gly Phe Ala Lys Arg Gly Leu
595 600 605

Gly Gln Gly Ala Ala His Ser Ser Leu Asn Trp Met Arg Arg Gly Ser
610 615 620

Thr Leu Leu Pro Thr Gly Cys
625 630

<210> 128

<211> 394

<212> PRT

<213> Aspergillus niger

<400> 128

Met Val Val Phe Ser Lys Thr Ala Ala Leu Val Leu Gly Leu Ser Ser
1 5 10 15

Ala Val Ser Ala Ala Pro Ala Pro Thr Arg Lys Gly Phe Thr Ile Asn
20 25 30

Gln Ile Ala Arg Pro Ala Asn Lys Thr Arg Thr Ile Asn Leu Pro Gly
 35 40 45

Met Tyr Ala Arg Ser Leu Ala Lys Phe Gly Gly Thr Val Pro Gln Ser
 50 55 60

Val Lys Glu Ala Ala Ser Lys Gly Ser Ala Val Thr Thr Pro Gln Asn
 65 70 75 80

Asn Asp Glu Glu Tyr Leu Thr Pro Val Thr Val Gly Lys Ser Thr Leu
 85 90 95

His Leu Asp Phe Asp Thr Gly Ser Ala Asp Leu Trp Val Phe Ser Asp
 100 105 110

Glu Leu Pro Ser Ser Glu Gln Thr Gly His Asp Leu Tyr Thr Pro Ser
 115 120 125

Ser Ser Ala Thr Lys Leu Ser Gly Tyr Thr Trp Asp Ile Ser Tyr Gly
 130 135 140

Asp Gly Ser Ser Ala Ser Gly Asp Val Tyr Arg Asp Thr Val Thr Val
 145 150 155 160

Gly Gly Val Thr Thr Asn Lys Gln Ala Val Glu Ala Ala Ser Lys Ile
 165 170 175

Ser Ser Glu Phe Val Gln Asn Thr Ala Asn Asp Gly Leu Leu Gly Leu
 180 185 190

Ala Phe Ser Ser Ile Asn Thr Val Gln Pro Lys Ala Gln Thr Thr Phe
 195 200 205

Phe Asp Thr Val Lys Ser Gln Leu Asp Ser Pro Leu Phe Ala Val Gln
 210 215 220

Leu Lys His Asp Ala Pro Gly Val Tyr Asp Phe Gly Tyr Ile Asp Asp
 225 230 235 240

Ser Lys Tyr Thr Gly Ser Ile Thr Tyr Thr Asp Ala Asp Ser Ser Gln
 245 250 255

Gly Tyr Trp Gly Phe Ser Thr Asp Gly Tyr Ser Ile Gly Asp Gly Ser
 260 265 270

Ser Ser Ser Ser Gly Phe Ser Ala Ile Ala Asp Thr Gly Thr Thr Leu
 275 280 285

Ile Leu Leu Asp Asp Glu Ile Val Ser Ala Tyr Tyr Glu Gln Val Ser
 290 295 300

Gly Ala Gln Glu Ser Glu Glu Ala Gly Gly Tyr Val Phe Ser Cys Ser
 305 310 315 320

Thr Asn Pro Pro Asp Phe Thr Val Val Ile Gly Asp Tyr Lys Ala Val
 325 330 335

Val Pro Gly Lys Tyr Ile Asn Tyr Ala Pro Ile Ser Thr Gly Ser Ser
 340 345 350

Thr Cys Phe Gly Gly Ile Gln Ser Asn Ser Gly Leu Gly Leu Ser Ile
 355 360 365

Leu Gly Asp Val Phe Leu Lys Ser Gln Tyr Val Val Phe Asn Ser Glu
 370 375 380

Gly Pro Lys Leu Gly Phe Ala Ala Gln Ala
 385 390

<210> 129

<211> 398

<212> PRT

<213> Aspergillus niger

<400> 129

Met Lys Ser Ala Ser Leu Leu Thr Ala Ser Val Leu Leu Gly Cys Ala
 1 5 10 15

Ser Ala Glu Val His Lys Leu Lys Leu Asn Lys Val Pro Leu Glu Glu
 20 25 30

Gln Leu Tyr Thr His Asn Ile Asp Ala His Val Arg Ala Leu Gly Gln
 35 40 45

Lys Tyr Met Gly Ile Arg Pro Ser Ile His Lys Glu Leu Val Glu Glu

50	55	60																	
Asn	Pro	Ile	Asn	Asp	Met	Ser	Arg	His	Asp	Val	Leu	Val	Asp	Asn	Phe				
65					70					75					80				
Leu	Asn	Ala	Gln	Tyr	Phe	Ser	Glu	Ile	Glu	Leu	Gly	Thr	Pro	Pro	Gln				
				85					90					95					
Lys	Phe	Lys	Val	Val	Leu	Asp	Thr	Gly	Ser	Ser	Asn	Leu	Trp	Val	Pro				
			100					105					110						
Ser	Ser	Glu	Cys	Ser	Ser	Ile	Ala	Cys	Tyr	Leu	His	Asn	Lys	Tyr	Asp				
		115					120					125							
Ser	Ser	Ala	Ser	Ser	Thr	Tyr	His	Lys	Asn	Gly	Ser	Glu	Phe	Ala	Ile				
	130					135					140								
Lys	Tyr	Gly	Ser	Gly	Ser	Leu	Ser	Gly	Phe	Ile	Ser	Gln	Asp	Thr	Leu				
145					150				155						160				
Lys	Ile	Gly	Asp	Leu	Lys	Val	Lys	Gly	Gln	Asp	Phe	Ala	Glu	Ala	Thr				
			165					170						175					
Asn	Glu	Pro	Gly	Leu	Ala	Phe	Ala	Phe	Gly	Arg	Phe	Asp	Gly	Ile	Leu				
		180						185					190						
Gly	Leu	Gly	Tyr	Asp	Thr	Ile	Ser	Val	Asn	Lys	Ile	Val	Pro	Pro	Phe				
	195						200					205							
Tyr	Asn	Met	Leu	Asp	Gln	Gly	Leu	Leu	Asp	Glu	Pro	Val	Phe	Ala	Phe				
	210					215					220								
Tyr	Leu	Gly	Asp	Thr	Asn	Lys	Glu	Gly	Asp	Glu	Ser	Val	Ala	Thr	Phe				
225					230				235						240				
Gly	Gly	Val	Asp	Lys	Asp	His	Tyr	Thr	Gly	Glu	Leu	Ile	Lys	Ile	Pro				
			245						250				255						
Leu	Arg	Arg	Lys	Ala	Tyr	Trp	Glu	Val	Glu	Leu	Asp	Ala	Ile	Ala	Leu				
		260					265						270						
Gly	Asp	Asp	Val	Ala	Glu	Met	Glu	Asn	Thr	Gly	Val	Ile	Leu	Asp	Thr				
	275					280						285							

Gly Thr Ser Leu Ile Ala Leu Pro Ala Asp Leu Ala Glu Met Ile Asn
 290 295 300

Ala Gln Ile Gly Ala Lys Lys Gly Trp Thr Gly Gln Tyr Thr Val Asp
 305 310 315 320

Cys Asp Lys Arg Ser Ser Leu Pro Asp Val Thr Phe Thr Leu Ala Gly
 325 330 335

His Asn Phe Thr Ile Ser Ser Tyr Asp Tyr Thr Leu Glu Val Gln Gly
 340 345 350

Ser Cys Val Ser Ala Phe Met Gly Met Asp Phe Pro Glu Pro Val Gly
 355 360 365

Pro Leu Ala Ile Leu Gly Asp Ala Phe Leu Arg Lys Trp Tyr Ser Val
 370 375 380

Tyr Asp Leu Gly Asn Ser Ala Val Gly Leu Ala Lys Ala Lys
 385 390 395

<210> 130
 <211> 393
 <212> PRT
 <213> Aspergillus niger

<400> 130

Met Arg Lys Tyr Arg Phe His Pro Thr Lys Pro Gly Pro Tyr Thr Leu
 1 5 10 15

Ser Ser Ser Ile Gln Gln Thr Gly Arg Pro Tyr Thr Glu Lys Pro Ile
 20 25 30

Gly Gly Arg Ala His Ile Arg Gln Leu Val Arg Lys Lys Ser Thr Thr
 35 40 45

Ser Asp Glu Val Gly Glu Val Pro Ala Glu Asp Val Gln Asn Asp Ser
 50 55 60

Met Tyr Leu Ala Thr Val Gly Ile Gly Thr Pro Ala Gln Asn Leu Lys
 65 70 75 80

Leu Asp Phe Asp Thr Gly Ser Ala Asp Leu Trp Val Trp Ser Asn Lys
 85 90 95

Leu Pro Ser Thr Leu Leu Ser Glu Asn Lys Thr His Ala Ile Phe Asp
 100 105 110

Ser Ser Lys Ser Ser Thr Phe Lys Thr Leu Glu Gly Glu Ser Trp Gln
 115 120 125

Ile Ser Tyr Gly Asp Gly Ser Ser Ala Ser Gly Ser Val Gly Thr Asp
 130 135 140

Asp Val Asn Ile Gly Gly Val Val Val Lys Asn Gln Ala Val Glu Leu
 145 150 155 160

Ala Glu Lys Met Ser Ser Thr Phe Ala Gln Gly Glu Gly Asp Gly Leu
 165 170 175

Leu Gly Leu Ala Phe Ser Asn Ile Asn Thr Val Gln Pro Lys Ser Val
 180 185 190

Lys Thr Pro Val Glu Asn Met Ile Leu Gln Asp Asp Ile Pro Lys Ser
 195 200 205

Ala Glu Leu Phe Thr Ala Lys Leu Asp Thr Trp Arg Asp Thr Asp Asp
 210 215 220

Glu Ser Phe Tyr Thr Phe Gly Phe Ile Asp Gln Asp Leu Val Lys Thr
 225 230 235 240

Ala Gly Glu Glu Val Tyr Tyr Thr Pro Val Asp Asn Ser Gln Gly Phe
 245 250 255

Trp Leu Phe Asn Ser Thr Ser Ala Thr Val Asn Gly Lys Thr Ile Asn
 260 265 270

Arg Ser Gly Asn Thr Ala Ile Ala Asp Thr Gly Thr Thr Leu Ala Leu
 275 280 285

Val Asp Asp Asp Thr Cys Glu Ala Ile Tyr Ser Ala Ile Asp Gly Ala
 290 295 300

Tyr Tyr Asp Gln Glu Val Gln Gly Trp Ile Tyr Pro Thr Asp Thr Ala

305 310 315 320
 Gln Asp Lys Leu Pro Thr Val Ser Phe Ala Val Gly Glu Lys Gln Phe
 325 330 335
 Val Val Gln Lys Glu Asp Leu Ala Phe Ser Glu Ala Lys Thr Gly Tyr
 340 345 350
 Val Tyr Gly Gly Ile Gln Ser Arg Gly Asp Met Thr Met Asp Ile Leu
 355 360 365
 Gly Asp Thr Phe Leu Lys Ser Ile Tyr Ala Val Ser Ala Leu Leu Leu
 370 375 380
 Ala Leu Arg Gly Asp Ile Glu Ala His
 385 390

 <210> 131
 <211> 282
 <212> PRT
 <213> Aspergillus niger

 <400> 131

 Met Lys Phe Ser Thr Ile Leu Thr Gly Ser Leu Phe Ala Thr Ala Ala
 1 5 10 15

 Leu Ala Ala Pro Leu Thr Glu Lys Arg Arg Ala Arg Lys Glu Ala Arg
 20 25 30

 Ala Ala Gly Lys Arg His Ser Asn Pro Pro Tyr Ile Pro Gly Ser Asp
 35 40 45

 Lys Glu Ile Leu Lys Leu Asn Gly Thr Ser Asn Glu Asp Tyr Ser Ser
 50 55 60

 Asn Trp Ala Gly Ala Val Leu Ile Gly Asp Gly Tyr Thr Lys Val Thr
 65 70 75 80

 Gly Glu Phe Thr Val Pro Ser Val Ser Ala Gly Ser Ser Ser Ser Ser
 85 90 95

 Gly Tyr Gly Gly Gly Tyr Gly Tyr Tyr Lys Asn Lys Arg Gln Ser Glu
 100 105 110

Glu Tyr Cys Ala Ser Ala Trp Val Gly Ile Asp Gly Asp Thr Cys Glu
 115 120 125

Thr Ala Ile Leu Gln Thr Gly Val Asp Phe Cys Tyr Glu Asp Gly Gln
 130 135 140

Thr Ser Tyr Asp Ala Trp Tyr Glu Trp Tyr Pro Asp Tyr Ala Tyr Asp
 145 150 155 160

Phe Asn Asp Ile Thr Ile Ser Glu Gly Asp Thr Ile Lys Val Thr Val
 165 170 175

Glu Ala Thr Ser Lys Ser Ser Gly Ser Ala Thr Val Glu Asn Leu Thr
 180 185 190

Thr Gly Gln Ser Val Thr His Thr Phe Ser Gly Asn Val Glu Gly Asp
 195 200 205

Leu Cys Glu Thr Asn Ala Glu Trp Ile Val Glu Asp Phe Glu Ser Gly
 210 215 220

Asp Ser Leu Val Ala Phe Ala Asp Phe Gly Ser Val Thr Phe Thr Asn
 225 230 235 240

Ala Glu Ala Thr Ser Asp Gly Ser Thr Val Gly Pro Ser Asp Ala Thr
 245 250 255

Val Met Asp Ile Glu Gln Asp Gly Thr Val Leu Thr Glu Thr Ser Val
 260 265 270

Ser Gly Asp Ser Val Thr Val Thr Tyr Val
 275 280

<210> 132

<211> 273

<212> PRT

<213> Aspergillus niger

<400> 132

Met Gly Asp Tyr Gly Pro Gly Val Ser Ser Leu Thr Ala Gln Leu Pro
 1 5 10 15

Gly Asn Pro Pro Val Ser Glu Thr Asp Gln Asp Glu Ile Ser Val Leu

20	25	30
Val Thr Gly Phe Gly Pro Phe Lys Ser Asn Leu Val Asn Ala Ser Tyr		
35	40	45
Leu Ile Ala Ser Ser Leu Pro Pro Ser Phe Thr Phe Ser Pro Ala Ser		
50	55	60
Ser Asp Gly Ser Asp Ala Val Pro Arg Arg Val Ser Ile Asn Val His		
65	70	75
Pro Ser Pro Ile Pro Val Ala Tyr Ser Ser Val Arg Thr Thr Leu Pro		
85	90	95
Val Ile Leu Asp Asp Tyr Ala Lys Thr His Gly Gly Arg Arg Pro Asp		
100	105	110
Ile Val Ile His Ile Gly Ile Ala Ala Met Arg Asn Tyr Tyr Ser Val		
115	120	125
Glu Thr Gln Ala His Arg Asp Gly Tyr Leu Met Ser Asp Ile Lys Gly		
130	135	140
Arg Ser Gly Tyr Glu Asp Gly Glu Lys Leu Trp Arg Glu Leu Asp Leu		
145	150	155
Pro Leu Val Leu Arg Ala Gly Pro Ser Glu Gly His Ala Ser Glu Lys		
165	170	175
Lys His Leu Ser Pro Arg Pro Pro Asp Glu Asp Phe Leu Ala Ala Trp		
180	185	190
Lys Thr Phe Cys Pro Pro Glu Thr Asp Ala Arg Ile Ser Thr Asp Ala		
195	200	205
Gly Arg Tyr Leu Cys Glu Phe Ile Leu Tyr Thr Ser Leu Ala Leu Ala		
210	215	220
Tyr Gln Ala Gly Glu Asp Arg Asn Val Thr Phe Phe His Val Pro Ala		
225	230	235
Ser Cys Leu Asp Glu Asp Ile Glu Thr Gly Lys Glu Val Ala Val Ala		
245	250	255

Leu Ile Lys Ala Leu Val Thr Ser Trp Ser Glu Gln Gln His Ser Val
 260 265 270

Pro

<210> 133
 <211> 542
 <212> PRT
 <213> *Aspergillus niger*

<400> 133

Met Gly Ser Arg Gln Gly Lys Ala Pro Phe Gly Trp Gly Thr Gln Ser
 1 5 10 15

Leu Ala His Phe Gly Ile Asn Pro Asp Leu Gly Leu His Asn Gln Gln
 20 25 30

Asn Leu Asn Ser Leu Ile Ser His Ser Ala Met Ala Thr Ala Leu Glu
 35 40 45

Thr Glu Tyr Ala Thr Ile Pro Ile Asp His Asn Asn Ala Ser Ala Gly
 50 55 60

Thr Tyr Gln Asn Arg Phe Trp Val Ser Asp Glu Phe Tyr Gln Pro Gly
 65 70 75 80

Asn Pro Ile Phe Val Tyr Asp Thr Gly Glu Ser Asp Gly Gly Ser Ile
 85 90 95

Ala Gln Ser Tyr Leu Thr Ser Thr Leu Ser Phe Phe Arg Glu Phe Leu
 100 105 110

Ile Glu Phe Asn Ala Met Gly Ile Ala Trp Glu His Arg Tyr Tyr Gly
 115 120 125

Asn Ser Thr Pro Ala Pro Val Ser Tyr Glu Thr Pro Pro Glu Ala Trp
 130 135 140

Gln Tyr Leu Thr Thr Lys Gln Ala Leu Ala Asp Leu Pro Tyr Phe Ala
 145 150 155 160

Ser Asn Phe Ser Arg Glu Lys Tyr Pro Asp Met Asp Leu Thr Pro Gln
 165 170 175
 Gly Thr Pro Trp Ile Met Val Gly Gly Ser Tyr Ala Gly Ile Arg Ala
 180 185 190
 Ala Leu Thr Arg Lys Glu Tyr Pro Glu Thr Ile Phe Ala Ala Phe Ser
 195 200 205
 Ser Ser Ser Pro Val Glu Ala Gln Val Asn Met Ser Ala Tyr Tyr Asp
 210 215 220
 Gln Val Tyr Arg Gly Met Val Ala Ser Gly Trp Thr Asn Cys Ser Ala
 225 230 235 240
 Asp Ile His Ala Ala Leu Glu Tyr Ile Asp Asp Gln Leu Ser Asp Glu
 245 250 255
 Asp Thr Ala Thr Ser Val Lys Gln Leu Phe Phe Gly Ser Gly Ala Glu
 260 265 270
 Thr Asn Ser Asn Gly Asp Phe Thr Ala Ala Leu Thr Ala Ile Tyr Gly
 275 280 285
 Tyr Phe Gln Ser Tyr Gly Met Ala Gly Gly Ile Gly Gly Leu Gly Ala
 290 295 300
 Phe Cys Glu Tyr Leu Glu Ile Asp Pro Lys Thr Asn Gly Thr Thr Gly
 305 310 315 320
 Pro Asp Gly Leu Ala Pro Thr Tyr Gly Gly Gln Tyr Val Ala Glu Arg
 325 330 335
 Trp Ala Ala Trp Pro Thr Phe Leu Glu Leu Val Asn Leu Asn Met Gly
 340 345 350
 Thr Asn Cys Gly Pro Gln Asp Ala Ser Gln Pro Ile Asp Cys Asp Phe
 355 360 365
 Ser Lys Pro Tyr Gly Asp Pro Ser Ala Ile Thr Trp Thr Trp Gln Tyr
 370 375 380
 Cys Ser Glu Trp Gly Phe Phe Gln Ala Asn Asn Asp Gly Pro His Ser

385 390 395 400
 Leu Ala Ser Arg Tyr Gln Ser Val Glu Tyr Gln Gln Glu Val Cys Asn
 405 410 415
 Arg Gln Phe Pro Asp Ala Val Asp Lys Gly Leu Leu Pro Pro Ser Pro
 420 425 430
 Arg Ala Asp Asp Val Asn Gln Glu Phe Gly Gly Trp Thr Ile Arg Pro
 435 440 445
 Ser Asn Val Tyr Phe Ser Gly Gly Glu Phe Asp Pro Trp Arg Ser Leu
 450 455 460
 Ser Ile Leu Ser Thr Glu Asp Phe Ala Pro Gln Gly Val Glu Phe Thr
 465 470 475 480
 Ser Ala Ile Pro Ala Cys Gly Val Gln Thr Asn Glu Asp Thr Val Phe
 485 490 495
 Gly Tyr Val Met Gln Asn Ser Glu His Cys Phe Asp Phe Gln Ala Thr
 500 505 510
 Pro Thr Val Gly Lys Leu Ser Arg Gly Ile Phe Thr Ser Ala Leu Leu
 515 520 525
 Gln Trp Leu Glu Cys Phe Gly Gln Asn Ser Ser Gln Ser Arg
 530 535 540

 <210> 134
 <211> 391
 <212> PRT
 <213> Aspergillus niger

 <400> 134
 Met Lys Leu Ser Ile Ala Leu Ala Leu Gly Ala Thr Ala Ser Thr Gly
 1 5 10 15

 Val Leu Ala Ala Val Val Pro Gln Gln Glu Pro Leu Ile Thr Pro Gln
 20 25 30

 Asp Pro Pro Thr His His His Gln Glu Lys Phe Leu Ile Glu Leu Ala
 35 40 45

Pro Tyr Gln Thr Arg Trp Val Thr Glu Glu Glu Lys Trp Asp Leu Lys
 50 55 60

Leu Asp Gly Val Asn Phe Ile Asp Ile Thr Glu Glu Arg Asn Thr Gly
 65 70 75 80

Phe Tyr Pro Thr Leu His Ala Gly Ser Tyr Val His Tyr Pro Pro Thr
 85 90 95

Met Lys His Ala Glu Lys Val Val Pro Leu Leu Arg Gly Leu Ser Lys
 100 105 110

Asp Asn Met Glu Gln Asn Leu Asn Lys Phe Thr Ser Phe His Thr Arg
 115 120 125

Tyr Tyr Arg Ser Ser Thr Gly Ile Glu Ser Ala Lys Trp Leu Tyr Ser
 130 135 140

Arg Val Ser Asp Val Ile Glu Gln Ser Gly Ala Ala Glu Tyr Gly Ala
 145 150 155 160

Thr Val Glu Gln Phe Ala His Ser Trp Gly Gln Phe Ser Ile Ile Ala
 165 170 175

Arg Ile Pro Gly Gln Thr Asn Lys Thr Val Val Leu Gly Ala His Gln
 180 185 190

Asp Ser Ile Asn Leu Phe Leu Pro Ser Ile Leu Ala Ala Pro Gly Ala
 195 200 205

Asp Asp Asp Gly Ser Gly Thr Val Thr Ile Leu Glu Ala Leu Arg Gly
 210 215 220

Leu Leu Gln Ser Asp Ala Ile Val Arg Gly Asn Ala Ser Asn Thr Ile
 225 230 235 240

Glu Phe His Trp Tyr Ser Ala Glu Glu Gly Gly Met Leu Gly Ser Gln
 245 250 255

Ala Ile Phe Ser Gln Tyr Lys Arg Asp Lys Arg Asp Ile Lys Ala Met
 260 265 270

Leu Gln Gln Asp Met Thr Gly Tyr Thr Gln Gly Ala Leu Asp Ala Gly
 275 280 285

Arg Gln Glu Ala Ile Gly Ile Met Val Asp Tyr Val Asp Glu Gly Leu
 290 295 300

Thr Gln Phe Leu Lys Asp Val Thr Thr Glu Tyr Cys Gly Ile Gly Tyr
 305 310 315 320

Ile Glu Thr Arg Cys Gly Tyr Ala Cys Ser Asp His Thr Ser Ala Ser
 325 330 335

Lys Tyr Gly Tyr Pro Ala Ala Met Ala Thr Glu Ser Glu Met Glu Asn
 340 345 350

Ser Asn Lys Arg Ile His Thr Thr Asp Asp Ser Ile Arg Tyr Leu Ser
 355 360 365

Phe Asp His Met Leu Glu His Ala Arg Leu Thr Leu Gly Phe Ala Tyr
 370 375 380

Glu Leu Ala Phe Ala Gln Phe
 385 390

<210> 135
 <211> 442
 <212> PRT
 <213> Aspergillus niger

<400> 135

Met Arg Thr Thr Thr Ser Phe Ala Arg Leu Ala Leu Ala Val Ala Ser
 1 5 10 15

Val Gly Ile Val Phe Ala Ser Pro Thr Lys Asn Asn Asp Gly Lys Leu
 20 25 30

Val Tyr Gly Ser Pro Glu Ser Val Gly Met Ile Ser Ala Pro Leu His
 35 40 45

Gln Met Val Gln Asn Val Ser Ala Tyr Thr His Ala Ala Asn Tyr Ser
 50 55 60

Lys Phe Ser Tyr Asp Lys Val His Pro Ile Glu Pro Gly Ser Val Thr
 65 70 75 80

Leu Val Ala Leu Asp Gly Val Ile Val Ser Glu Phe Ala Leu Gly Lys
85 90 95

Arg Asn Leu Tyr Ala Asp Val Asn Gly Thr Asn Leu Pro Arg Tyr Leu
100 105 110

Gln Glu Asp Thr Thr Leu Asp Thr Val Tyr Asp Met Ala Ser Leu Thr
115 120 125

Lys Leu Phe Thr Thr Val Ala Ala Leu Arg Glu Leu Asp Ala Gly Arg
130 135 140

Ile Ala Leu Asn Val Thr Val Ala Thr Tyr Ile Pro Asp Phe Ala Thr
145 150 155 160

Asn Gly Lys Glu Asn Ile Thr Ile Leu Glu Leu Phe Thr His Thr Ser
165 170 175

Gly Phe Ala Ser Asp Pro Ser Pro Pro Leu Phe Ser Ala Tyr Tyr Thr
180 185 190

Thr Tyr Asp Glu Arg Ile Lys Ala Ile Leu Thr Gln Lys Ile Ile Asn
195 200 205

Thr Pro Gly Ser Thr Tyr Leu Tyr Leu Asp Leu Asn Phe Met Ser Leu
210 215 220

Gly Leu Val Ile Glu Thr Val Thr Gly Arg Ala Leu Asp Asp Leu Ile
225 230 235 240

Tyr Asp Phe Thr Arg Pro Leu Glu Met Thr Ser Thr Phe Phe Asn Arg
245 250 255

Gly Asn Ile Glu Gly Ser Thr Pro Gln Ser Pro Asn Tyr Asp Arg Thr
260 265 270

Ala Val Gln Glu Phe Gln Ile Ala Ala Leu Gly Pro Ser Glu Pro Gln
275 280 285

Arg Pro Gln Pro Val Arg Gly Thr Val His Asp Glu Asn Ala Trp Ser
290 295 300

Leu Asp Gly Val Ser Gly His Ala Gly Leu Phe Ser Thr Val Arg Asp
 305 310 315 320

Thr Ala Thr Phe Cys Gln Met Ile Leu Asn Asn Gly Thr Tyr Ala Gly
 325 330 335

Gln Arg Ile Leu Ser Arg Thr Ala Val Asp Met Ile Phe Thr Asn Phe
 340 345 350

Asn Ala Arg Phe Pro Gly Asp Ala Arg Ser Leu Gly Phe Glu Leu Asp
 355 360 365

Gln Tyr Ser Thr Ala Gly Pro Met Ala Ser Leu Gln Thr Ala Ser His
 370 375 380

Thr Gly Phe Thr Gly Thr Thr Leu Val Met Asp Arg Thr Tyr Asn Ala
 385 390 395 400

Phe Trp Leu His Phe Ser Asn Arg Val His Pro Ser Arg Ala Trp Ser
 405 410 415

Ser Asn Thr Ile Val Arg Glu Ala Ile Gly Tyr Trp Val Gly Lys Ser
 420 425 430

Leu Gly Leu Asp Val Ala Phe Ala Leu Leu
 435 440

<210> 136
 <211> 612
 <212> PRT
 <213> Aspergillus niger

<400> 136

Met Ala Ser Trp Leu Leu Ser Thr Leu Leu Phe Leu Ser Pro Ser Leu
 1 5 10 15

Val Ser Ala Lys Ser Ala Ala Asp Tyr Tyr Val His Ser Leu Pro Gly
 20 25 30

Ala Pro Glu Gly Pro Leu Leu Lys Met His Ala Gly His Ile Glu Val
 35 40 45

Asp Pro Gln Asn Asn Gly Asn Leu Phe Phe Trp His Tyr Gln Asn Arg

50	55	60
His Ile Ala Asn Arg Gln Arg Thr Val Ile Trp Leu Asn Gly Gly Pro		
65	70	75 80
Gly Cys Ser Ser Met Asp Gly Ala Leu Met Glu Val Gly Pro Tyr Arg		
	85	90 95
Leu Lys Asp Asn Glu Thr Leu Thr Tyr Asn Glu Gly Ser Trp Asp Glu		
	100	105 110
Phe Ala Asn Leu Leu Phe Val Asp Gln Pro Val Gly Thr Gly Phe Ser		
	115	120 125
Tyr Val Asn Thr Asp Ser Tyr Leu His Glu Leu Asp Glu Met Ser Ala		
	130	135 140
Gln Phe Ile Val Phe Leu Glu Glu Trp Phe Arg Leu Phe Pro Glu Tyr		
	145	150 155 160
Glu Arg Asp Asp Ile Tyr Ile Ala Gly Glu Ser Tyr Ala Gly Gln His		
	165	170 175
Ile Pro Tyr Ile Ala Lys Ala Ile Gln Glu Arg Asn Lys Asn Val Gln		
	180	185 190
Gly Lys Thr Ile Ala Ser Trp Asn Leu Lys Gly Leu Leu Ile Gly Asn		
	195	200 205
Gly Trp Ile Ser Pro Asn Glu Gln Tyr Met Ser Tyr Leu Pro Tyr Ala		
	210	215 220
Tyr Glu Glu Gly Leu Ile Lys Glu Gly Ser Arg Thr Ala Lys Glu Leu		
	225	230 235 240
Glu Val Leu Gln Ser Val Cys Lys Ser Arg Leu Glu Thr Gly Lys Asn		
	245	250 255
Lys Val His Leu Asn Asp Cys Glu Lys Val Met Asn Ala Leu Leu Asp		
	260	265 270
Lys Thr Val Glu Asp Asn Lys Cys Leu Asn Met Tyr Asp Ile Arg Leu		
	275	280 285

Arg Asp Thr Thr Asp Ala Cys Gly Met Asn Trp Pro Thr Asp Leu Glu
 290 295 300

Asp Val Lys Pro Tyr Leu Gln Arg Glu Asp Val Val Lys Ala Leu Asn
 305 310 315 320

Ile Asn Pro Glu Lys Lys Ser Gly Trp Val Glu Cys Ser Gly Ala Val
 325 330 335

Ser Ser Ala Phe Asn Pro Gln Lys Ser Pro Pro Ser Val Gln Leu Leu
 340 345 350

Pro Gly Leu Leu Glu Ser Gly Leu Gln Ile Leu Leu Phe Ser Gly Asp
 355 360 365

Lys Asp Leu Ile Cys Asn His Val Gly Thr Glu Gln Leu Ile Asn Asn
 370 375 380

Met Lys Trp Asn Gly Gly Thr Gly Phe Glu Thr Ser Pro Gly Val Trp
 385 390 395 400

Ala Pro Arg His Asp Trp Ser Phe Glu Gly Glu Pro Ala Gly Ile Tyr
 405 410 415

Gln Tyr Ala Arg Asn Leu Thr Tyr Val Leu Ile Tyr Asn Ala Ser His
 420 425 430

Met Val Pro Tyr Asp Leu Pro Arg Gln Ser Arg Asp Met Leu Asp Arg
 435 440 445

Phe Met Asn Val Asp Ile Ala Ser Ile Gly Gly Ser Pro Ala Asp Ser
 450 455 460

Arg Ile Asp Gly Glu Lys Leu Pro Gln Thr Ser Val Gly Gly His Pro
 465 470 475 480

Asn Ser Thr Ala Ala Glu Glu Gln Glu Lys Glu Arg Ile Lys Glu Thr
 485 490 495

Glu Trp Lys Ala Tyr Ala Lys Ser Gly Glu Ala Val Leu Leu Val Val
 500 505 510

Ile Ile Gly Val Leu Val Trp Gly Phe Phe Ile Trp Arg Ser Arg Arg
 515 520 525

Arg His Gln Gly Tyr Arg Gly Val Trp His Lys Asp Met Ser Gly Ser
 530 535 540

Ser Val Leu Glu Arg Phe His Asn Lys Arg Thr Gly Gly Ala Asp Val
 545 550 555 560

Glu Ala Gly Asp Phe Asp Glu Ala Glu Leu Asp Asp Leu His Ser Pro
 565 570 575

Asp Leu Glu Arg Glu His Tyr Ala Val Gly Glu Asp Ser Asp Glu Asp
 580 585 590

Asp Ile Ser Arg Gln His Ser Gln Gln Ala Ser Arg Ala Gly Gly Ser
 595 600 605

His Asn Leu Ser
 610

<210> 137
 <211> 531
 <212> PRT
 <213> Aspergillus niger

<400> 137

Met Phe Leu Ile Ser Pro Ala Val Thr Val Ala Ala Ala Leu Leu Leu
 1 5 10 15

Ile Asn Gly Ala Gly Ala Thr Gln Ser Glu Arg Ser Arg Ala Ala Ala
 20 25 30

His Phe Ser Lys Arg His Pro Thr Tyr Arg Ala Ala Thr Arg Ala Gln
 35 40 45

Ser Ser Asn Thr Ser Asp Tyr Arg Phe Phe Asn Asn Arg Thr Lys Pro
 50 55 60

His Leu Val Glu Ser Leu Pro Asp Val His Phe Asp Val Gly Glu Met
 65 70 75 80

Tyr Ser Gly Ser Ile Pro Ile Asp Asp Ser Asn Asn Gly Ser Arg Ser

	85		90		95
Leu Phe Tyr Ile Phe Gln Pro Lys Ile Gly Glu Pro Ser Asp Asp Leu	100		105		110
Thr Ile Tyr Leu Asn Gly Gly Pro Gly Cys Ser Ser Glu Gln Gly Phe	115		120		125
Phe Gln Glu Asn Gly Arg Phe Thr Trp Gln Pro Gly Thr Tyr Ala Pro	130		135		140
Val Ile Asn Glu Tyr Ser Trp Val Asn Leu Thr Asn Met Leu Trp Val	145		150		155
Asp Gln Pro Val Gly Thr Gly Phe Ser Val Gly Asn Val Thr Ala Thr	165		170		175
Asn Glu Glu Glu Ile Ala Ala Asp Phe Leu Asp Phe Phe Glu Lys Phe	180		185		190
Glu Asp Leu Tyr Gly Ile Lys Asn Phe Arg Ile Phe Met Thr Gly Glu	195		200		205
Ser Tyr Ala Gly Arg Tyr Val Pro Tyr Ile Ser Ser Ala Met Leu Asp	210		215		220
Lys Asn Asp Thr Thr Arg Phe Asn Leu Ser Gly Ala Leu Leu Tyr Asp	225		230		235
Ala Cys Ile Gly Gln Trp Asp Tyr Ile Gln Ala Glu Leu Pro Ala Tyr	245		250		255
Pro Phe Val Lys Gln His Ala Ser Leu Phe Asn Phe Asn Gln Ser Tyr	260		265		270
Met Asn Glu Leu Glu Thr Thr Tyr Glu Glu Cys Gly Tyr Lys Ala Tyr	275		280		285
Phe Asp Glu Tyr Phe Ala Phe Pro Pro Ser Gly Ile Gln Pro Pro Lys	290		295		300
Tyr Met Asn Tyr Ser Glu Cys Asp Ile Tyr Asn Met Ile Tyr Tyr Glu	305		310		315
					320

Ala Tyr Asn Pro Asn Pro Cys Phe Asn Pro Tyr Arg Val Ile Asp Glu
325 330 335

Cys Pro Leu Leu Trp Asp Val Leu Gly Trp Pro Thr Asp Leu Ala Tyr
340 345 350

Glu Pro Ala Pro Thr Thr Tyr Phe Asn Arg Ile Asp Val Lys Lys Ala
355 360 365

Leu His Ala Pro Met Asp Val Glu Trp Glu Leu Cys Ser Tyr Asp Leu
370 375 380

Val Phe Ala Gly Gly Asp Ala Asp Pro Gly Pro Glu Gln Gln Gly Asp
385 390 395 400

Asp Ser Pro Asn Pro Thr Glu Gly Val Leu Pro Arg Val Ile Glu Ala
405 410 415

Thr Asn Arg Val Leu Ile Ala Asn Gly Asp Trp Asp Tyr Leu Ile Ile
420 425 430

Thr Asn Gly Thr Leu Leu Ala Ile Gln Asn Met Thr Trp Asn Gly Gln
435 440 445

Leu Gly Phe Gln Ser Ala Pro Ala Thr Pro Ile Asp Ile Gln Met Pro
450 455 460

Asp Leu Gln Trp Val Glu Ile Phe Glu Ala Gln Glu Gly Tyr Gly Gly
465 470 475 480

Leu Asp Gly Pro Gln Gly Val Met Gly Val Gln His Tyr Glu Arg Gly
485 490 495

Leu Met Trp Ala Glu Thr Tyr Gln Ser Gly His Lys Gln Ala Gln Asp
500 505 510

Gln Gly Arg Val Ser Tyr Arg His Leu Gln Trp Leu Leu Gly Gln Val
515 520 525

Glu Ile Leu
530

<210> 138
 <211> 531
 <212> PRT
 <213> *Aspergillus niger*

<400> 138

Met Leu Phe Arg Ser Leu Leu Ser Thr Ala Val Leu Ala Val Ser Leu
 1 5 10 15

Cys Thr Asp Asn Ala Ser Ala Ala Lys His Gly Arg Phe Gly Gln Lys
 20 25 30

Ala Arg Asp Ala Met Asn Ile Ala Lys Arg Ser Ala Asn Ala Val Lys
 35 40 45

His Ser Leu Lys Ile Pro Val Glu Asp Tyr Gln Phe Leu Asn Asn Lys
 50 55 60

Thr Lys Pro Tyr Arg Val Glu Ser Leu Pro Asp Val His Phe Asp Leu
 65 70 75 80

Gly Glu Met Tyr Ser Gly Leu Val Pro Ile Glu Lys Gly Asn Val Ser
 85 90 95

Arg Ser Leu Phe Phe Val Phe Gln Pro Thr Ile Gly Glu Pro Val Asp
 100 105 110

Glu Ile Thr Ile Trp Leu Asn Gly Gly Pro Gly Cys Ser Ser Leu Glu
 115 120 125

Ala Phe Leu Gln Glu Asn Gly Arg Phe Val Trp Gln Pro Gly Thr Tyr
 130 135 140

Gln Pro Val Glu Asn Pro Tyr Ser Trp Val Asn Leu Thr Asn Val Leu
 145 150 155 160

Trp Val Asp Gln Pro Val Gly Thr Gly Phe Ser Leu Gly Val Pro Thr
 165 170 175

Ala Thr Ser Glu Glu Glu Ile Ala Glu Asp Phe Val Lys Phe Phe Lys
 180 185 190

Asn Trp Gln Gln Ile Phe Gly Ile Lys Asn Phe Lys Ile Tyr Val Thr

195	200	205
Gly Glu Ser Tyr Ala Gly Arg Tyr Val Pro Tyr Ile Ser Ala Ala Phe 210 215 220		
Leu Asp Gln Asn Asp Thr Glu His Phe Asn Leu Lys Gly Ala Leu Ala 225 230 235 240		
Tyr Asp Pro Cys Ile Gly Gln Phe Asp Tyr Val Gln Glu Glu Ala Pro 245 250 255		
Val Val Pro Phe Val Gln Lys Asn Asn Ala Leu Phe Asn Phe Asn Ala 260 265 270		
Ser Phe Leu Ala Glu Leu Glu Ser Ile His Glu Gln Cys Gly Tyr Lys 275 280 285		
Asp Phe Ile Asp Gln Tyr Leu Val Phe Pro Ala Ser Gly Val Gln Pro 290 295 300		
Pro Lys Ala Met Asn Trp Ser Asp Pro Thr Cys Asp Val Tyr Asp Ile 305 310 315 320		
Val Asn Asn Ala Val Leu Asp Pro Asn Pro Cys Phe Asn Pro Tyr Glu 325 330 335		
Ile Asn Glu Met Cys Pro Ile Leu Trp Asp Val Leu Gly Phe Pro Thr 340 345 350		
Glu Val Asp Tyr Leu Pro Ala Gly Ala Ser Ile Tyr Phe Asp Arg Ala 355 360 365		
Asp Val Lys Arg Ala Met His Ala Pro Asn Ile Thr Trp Ser Glu Cys 370 375 380		
Ser Val Glu Ser Val Phe Val Gly Gly Asp Gly Gly Pro Glu Gln Glu 385 390 395 400		
Gly Asp Tyr Ser Ala Asn Pro Ile Glu His Val Leu Pro Gln Val Ile 405 410 415		
Glu Gly Thr Asn Arg Val Leu Ile Gly Asn Gly Asp Tyr Asp Met Val 420 425 430		

Ile Leu Thr Asn Gly Thr Leu Leu Ser Ile Gln Asn Met Thr Trp Asn
 435 440 445

Gly Lys Leu Gly Phe Asp Thr Ala Pro Ser Thr Pro Ile Asn Ile Asp
 450 455 460

Ile Pro Asp Leu Met Tyr Asn Glu Val Phe Ile Glu Asn Gly Tyr Asp
 465 470 475 480

Pro Gln Gly Gly Gln Gly Val Met Gly Ile Gln His Tyr Glu Arg Gly
 485 490 495

Leu Met Trp Ala Glu Thr Phe Gln Ser Gly His Met Gln Pro Gln Phe
 500 505 510

Gln Pro Arg Val Ser Tyr Arg His Leu Glu Trp Leu Leu Gly Arg Arg
 515 520 525

Asp Thr Leu
 530

<210> 139
 <211> 492
 <212> PRT
 <213> Aspergillus niger

<400> 139

Met Lys Gly Ala Ala Leu Ile Pro Leu Ala Ala Gly Ile Pro Phe Ala
 1 5 10 15

His Gly Leu Ser Leu His Lys Arg Asp Gly Pro Ala Val Val Arg Met
 20 25 30

Pro Ile Glu Arg Arg Ser Ala Gln Ser Leu Gln Lys Arg Asp Ser Thr
 35 40 45

Val Gly Val Thr Leu Gln Asn Trp Asp Ala Thr Tyr Tyr Ala Val Asn
 50 55 60

Leu Thr Leu Gly Thr Pro Ala Gln Lys Val Ser Leu Ala Leu Asp Thr
 65 70 75 80

Gly Ser Ser Asp Leu Trp Val Asn Thr Gly Asn Ser Thr Tyr Cys Ser
85 90 95

Ile Asp Asn Leu Cys Thr Pro Tyr Gly Leu Tyr Asn Ala Ser Glu/Ser
100 105 110

Ser Thr Val Lys Thr Val Gly Thr His Leu Asn Asp Thr Tyr Ala Asp
115 120 125

Gly Thr Asn Leu Tyr Gly Pro Tyr Val Thr Asp Lys Leu Thr Ile Gly
130 135 140

Asn Thr Thr Ile Asp Asn Met Gln Phe Gly Ile Ala Glu Ser Thr Thr
145 150 155 160

Ser Lys Arg Gly Ile Ala Gly Val Gly Tyr Lys Ile Ser Thr Tyr Gln
165 170 175

Ala Glu His Asp Asp Lys Val Tyr Ala Asn Leu Pro Gln Ala Leu Val
180 185 190

Asp Ser Gly Ala Ile Lys Ser Ala Ala Tyr Ser Ile Trp Leu Asp Ser
195 200 205

Leu Glu Ala Ser Thr Gly Ser Leu Leu Phe Gly Gly Val Asn Thr Ala
210 215 220

Lys Tyr Lys Gly Asp Leu Gln Thr Leu Pro Ile Ile Pro Val Tyr Gly
225 230 235 240

Lys Tyr Tyr Ser Leu Ala Ile Ala Leu Thr Glu Leu Ser Val Ala Thr
245 250 255

Asp Ser Asn Ser Ser Ser Phe Thr Asp Ser Leu Pro Leu Ser Val Ser
260 265 270

Leu Asp Thr Gly Thr Thr Met Thr Ala Leu Pro Ser Asp Leu Val Asn
275 280 285

Lys Val Tyr Asp Ala Leu Asn Ala Thr Tyr Asp Lys Thr Tyr Asp Met
290 295 300

Ala Tyr Ile Asp Cys Asp Thr Arg Glu Ala Asp Tyr Asn Val Thr Tyr

305 310 315 320
 Ser Phe Ser Gly Ala Thr Ile Thr Val Ser Met Ser Glu Leu Ile Ile
 325 330 335
 Pro Ala Thr Glu Pro Gly Trp Pro Asp Asn Thr Cys Val Leu Gly Leu
 340 345 350
 Val Pro Ser Gln Pro Gly Val Asn Leu Leu Gly Asp Thr Phe Leu Arg
 355 360 365
 Ser Ala Tyr Val Val Tyr Asp Leu Glu Asn Asn Glu Ile Ser Leu Ala
 370 375 380
 Asn Thr Asn Phe Asn Pro Gly Asp Asp Asp Ile Leu Glu Ile Gly Thr
 385 390 395 400
 Gly Thr Ser Ala Val Pro Gly Ala Thr Pro Val Pro Ser Ala Val Ser
 405 410 415
 Ser Ala Thr Gly Asn Gly Leu Ile Ser Ser Gly Thr Ala Val Pro Thr
 420 425 430
 Leu Ser Gly Val Thr Ile Thr Ala Thr Ala Thr Ala Thr Gly Ser Thr
 435 440 445
 Gly Thr Gly Ser Ser Gly Gly Ser Ser Ala Glu Ala Thr Ser Thr Ser
 450 455 460
 Ser Glu Gly Ala Ala Ala Gln Ala Thr Ser Asn Pro Met Asn Leu Leu
 465 470 475 480
 Pro Gly Leu Ala Gly Ile Gly Leu Leu Leu Ala Leu
 485 490

 <210> 140
 <211> 611
 <212> PRT
 <213> *Aspergillus niger*

 <400> 140
 Met Leu Ser Ser Leu Leu Ser Gln Gly Ala Ala Val Ser Leu Ala Val
 1 5 10 15

Leu Ser Leu Leu Pro Ser Pro Val Ala Ala Glu Ile Phe Glu Lys Leu
 20 25 30

Ser Gly Val Pro Asn Gly Trp Arg Tyr Ala Asn Asn Pro Gln Gly Asn
 35 40 45

Glu Val Ile Arg Leu Gln Ile Ala Leu Gln Gln His Asp Val Ala Gly
 50 55 60

Phe Glu Gln Ala Val Met Asp Met Ser Thr Pro Gly His Ala Asp Tyr
 65 70 75 80

Gly Lys His Phe Arg Thr His Asp Glu Met Lys Arg Met Leu Leu Pro
 85 90 95

Ser Glu Thr Ala Val Asp Ser Val Arg Asp Trp Leu Glu Ser Ala Gly
 100 105 110

Val His Asn Ile Gln Val Asp Ala Asp Trp Val Lys Phe His Thr Thr
 115 120 125

Val Asn Lys Ala Asn Ala Leu Leu Asp Ala Asp Phe Lys Trp Tyr Val
 130 135 140

Ser Asp Ala Lys His Ile Arg Arg Leu Arg Thr Leu Gln Tyr Ser Ile
 145 150 155 160

Pro Asp Ala Leu Val Ser His Ile Asn Met Ile Gln Pro Thr Thr Arg
 165 170 175

Phe Gly Gln Ile Gln Pro Asn Arg Ala Thr Met Arg Ser Lys Pro Lys
 180 185 190

His Ala Asp Glu Thr Phe Leu Thr Ala Ala Thr Leu Ala Gln Asn Thr
 195 200 205

Ser His Cys Asp Ser Ile Ile Thr Pro His Cys Leu Lys Gln Leu Tyr
 210 215 220

Asn Ile Gly Asp Tyr Gln Ala Asp Pro Lys Ser Gly Ser Lys Ile Gly
 225 230 235 240

Phe Ala Ser Tyr Leu Glu Glu Tyr Ala Arg Tyr Ala Asp Leu Glu Arg
 245 250 255

Phe Glu Gln His Leu Ala Pro Asn Ala Ile Gly Gln Asn Phe Ser Val
 260 265 270

Val Gln Phe Asn Gly Gly Leu Asn Asp Gln Leu Ser Ser Ser Asp Ser
 275 280 285

Gly Glu Ala Asn Leu Asp Leu Gln Tyr Ile Leu Gly Val Ser Ala Pro
 290 295 300

Val Pro Ile Thr Glu Tyr Ser Thr Gly Gly Arg Gly Glu Leu Val Pro
 305 310 315 320

Asp Leu Ser Ser Pro Asp Pro Asn Asp Asn Ser Asn Glu Pro Tyr Leu
 325 330 335

Asp Phe Leu Gln Gly Ile Leu Lys Leu Asn Asn Ser Asp Leu Pro Gln
 340 345 350

Val Ile Ser Thr Ser Tyr Gly Glu Asp Glu Gln Thr Ile Pro Val Pro
 355 360 365

Tyr Ala Arg Thr Val Cys Asn Leu Tyr Ala Gln Leu Gly Ser Arg Gly
 370 375 380

Val Ser Val Ile Phe Ser Ser Gly Asp Ser Gly Val Gly Ala Ala Cys
 385 390 395 400

Leu Thr Asn Asp Gly Thr Asn Arg Thr His Phe Pro Pro Gln Phe Pro
 405 410 415

Ala Ser Cys Pro Trp Val Thr Ser Val Gly Ala Thr Ser Lys Thr Ser
 420 425 430

Pro Glu Gln Ala Val Ser Phe Ser Ser Gly Gly Phe Ser Asp Leu Trp
 435 440 445

Pro Arg Pro Ser Tyr Gln His Ala Ala Val Gln Thr Tyr Leu Thr Lys
 450 455 460

His Leu Gly Asn Lys Phe Ser Gly Leu Phe Asn Ala Ser Gly Arg Ala

465 470 475 480
 Phe Pro Asp Val Ser Ala Gln Gly Val Asn Tyr Ala Val Tyr Asp Lys
 485 490 495
 Gly Met Leu Gly Gln Phe Asp Gly Thr Ser Cys Ser Ala Pro Thr Phe
 500 505 510
 Ser Gly Val Ile Ala Leu Leu Asn Asp Ala Arg Leu Arg Ala Gly Leu
 515 520 525
 Pro Val Met Gly Phe Leu Asn Pro Phe Leu Tyr Gly Val Gly Ser Glu
 530 535 540
 Lys Gly Ala Leu Asn Asp Ile Val Asn Gly Gly Ser Val Gly Cys Asp
 545 550 555 560
 Gly Arg Asn Arg Phe Gly Gly Thr Pro Asn Gly Ser Pro Val Val Pro
 565 570 575
 Phe Ala Ser Trp Asn Ala Thr Thr Gly Trp Asp Pro Val Ser Gly Leu
 580 585 590
 Gly Thr Pro Asp Phe Ala Lys Leu Lys Gly Val Ala Leu Gly Glu Glu
 595 600 605
 Gly Gly Asn
 610

 <210> 141
 <211> 478
 <212> PRT
 <213> Aspergillus niger

 <400> 141
 Met Trp Leu Phe Leu Val Cys Ser Ile Leu Leu Pro Leu Gly Val Val
 1 5 10 15
 Asn Ala Gln Ser Gln Tyr Phe Asn Asn Lys Thr Lys Glu Phe Val Val
 20 25 30
 Asn Gly Ser Ala Ile Pro Phe Val Asp Phe Asp Ile Gly Glu Ser Tyr
 35 40 45

Ala Gly Tyr Leu Pro Asn Thr Pro Ser Gly Ile Ser Ser Leu Tyr Phe
 50 55 60

Trp Phe Phe Pro Ser Ser Asp Pro Asp Ala Ser Asp Glu Ile Thr Val
 65 70 75 80

Trp Leu Asn Gly Gly Pro Gly Cys Ser Ser Leu Ala Gly Ile Met Leu
 85 90 95

Glu Asn Gly Pro Phe Leu Trp Gln Pro Gly Thr Tyr Arg Pro Val Arg
 100 105 110

Asn Pro Tyr Ala Trp Asn Asn Leu Thr Asn Met Val Tyr Ile Asp Gln
 115 120 125

Pro Ala Gly Thr Gly Phe Ser Leu Gly Pro Ser Thr Val Val Ser Glu
 130 135 140

Phe Asp Val Ala Arg Gln Phe Met Asp Phe Trp Arg Arg Phe Met Lys
 145 150 155 160

Thr Phe Asp Leu Gln Asn Arg Lys Ile Tyr Leu Thr Gly Glu Ser Tyr
 165 170 175

Ala Gly Gln Tyr Ile Pro Tyr Ile Ala Ser Gln Met Leu Asp Gln Asp
 180 185 190

Asp Asp Glu Tyr Phe Arg Val Ala Gly Ile Gln Ile Asn Asp Pro Tyr
 195 200 205

Ile Asn Glu Leu Pro Val Leu Gln Asp Val Ala Thr Val Asn Gln His
 210 215 220

Arg Ser Leu Phe Pro Phe Asn Asp Thr Phe Met Ser Gln Ile Thr Lys
 225 230 235 240

Leu Ser Asp Asp Cys Gly Tyr Thr Ser Phe Leu Asp Asp Ala Leu Thr
 245 250 255

Phe Pro Pro Arg Ser Gln Phe Pro Ser Val Pro Tyr Asn Ala Ser Cys
 260 265 270

Asn Ile Trp Asp Ile Ile Asn Asn Ala Ser Leu Ala Leu Asn Pro Cys
 275 280 285

Phe Asn Arg Tyr His Ile Pro Asp Ala Cys Pro Thr Pro Trp Asn Pro
 290 295 300

Val Gly Gly Pro Ile Val Gly Leu Gly Pro Thr Asn Tyr Phe Asn Arg
 305 310 315 320

Ser Asp Val Gln Lys Ala Ile Asn Ala Tyr Pro Thr Asp Tyr Phe Val
 325 330 335

Cys Lys Asp Gly Ile Phe Pro Thr Ala Asn Gly Leu Asp Thr Ser Pro
 340 345 350

Pro Ser Ser Leu Gly Pro Leu Pro Arg Val Ile Glu Gln Thr Asn Asn
 355 360 365

Thr Ile Ile Ala His Gly Leu Met Asp Phe Glu Leu Leu Ala Gln Gly
 370 375 380

Thr Leu Ile Ser Ile Gln Asn Met Thr Trp Asn Gly Lys Gln Gly Phe
 385 390 395 400

Glu Arg Glu Pro Val Glu Pro Leu Phe Val Pro Tyr Gly Gly Ser Ser
 405 410 415

Gly Gly Gly Val Leu Gly Thr Ala His Thr Glu Arg Gly Leu Thr Phe
 420 425 430

Ser Thr Val Phe Ser Ser Gly His Glu Ile Pro Glu Tyr Ala Pro Gly
 435 440 445

Ala Ala Tyr Arg Gln Leu Glu Phe Leu Leu Gly Arg Val Ala Asn Leu
 450 455 460

Ser Thr Ile Ile Glu Gln Val Gln Ile Thr Glu Gln Asn Gly
 465 470 475

<210> 142

<211> 210

<212> PRT

<213> Aspergillus niger

<400> 142

Met Ser Lys Leu Ser Ala Ala Ile Ser Lys Leu Ser Leu Ser Thr Ile
 1 5 10 15

Ala Thr Thr Leu Leu Leu Thr Pro Pro Thr Thr Ala Tyr Phe Tyr
 20 25 30

Lys Tyr Pro Ala Leu Phe Val Tyr Lys Asp Thr Asn Cys Thr Asp Ile
 35 40 45

Ser Phe Ser Leu Val Tyr Pro Ser Leu Gly Asn Cys Asn Gly Gly Tyr
 50 55 60

Tyr Asp Tyr Ala Gly Ser Phe Gln Met Phe Asn Ile Asp Ala Ala Tyr
 65 70 75 80

Thr Cys Asn Gly Ser Asp Ser Thr Leu Met Phe Glu Met Tyr Asn Ser
 85 90 95

Ser Gly Ser Asp Cys Gly Asp Glu Ser Asp Leu Leu Phe Arg Gln Pro
 100 105 110

Val Thr Glu Glu Cys Thr Val Ala Asp Val Glu Ser Pro Gly Pro Leu
 115 120 125

Glu Met Pro Val Trp Phe Glu Leu Gly Ser Leu Leu Gly Asn Cys Gly
 130 135 140

Gly Met Ala Gly Thr Met Leu Phe Gly Val Gly Ile Leu Glu Gly Gly
 145 150 155 160

Leu Glu Thr Lys Leu Tyr Trp Lys Cys Tyr Ser Ser Arg Leu Asn Thr
 165 170 175

Ser Val Thr Val His Arg Leu Ser Leu Ile Leu Ser Met Gly Cys Thr
 180 185 190

Ser Val Ser Asp Ser Tyr Asn Glu Leu Ala Ala Ala His Tyr Tyr Glu
 195 200 205

Asp Leu
 210

<210> 143
 <211> 608
 <212> PRT
 <213> *Aspergillus niger*

<400> 143

Met Arg His Leu Leu Ser Leu Leu Val Leu Leu Ile Ala Ser Ala Ala
 1 5 10 15

Leu Val Ser Ala Val Pro Ala Gly Ser Ile Ile Thr Pro Gln Pro Pro
 20 25 30

Val Glu Pro Val His Leu Leu Ser Ser Gln Pro Ser Asp Pro Arg Arg
 35 40 45

Pro Trp Ile Arg Leu Arg Asp Trp Ile Ile Glu Ser Ile Trp Gly Ile
 50 55 60

Glu Lys Pro Ala Ser Arg Arg Phe Pro Leu Asn Asp Ser Pro Arg Asn
 65 70 75 80

Arg Ser Pro Pro Ser Arg Ile Leu Ala Arg Tyr Gly Ser Asp Val Val
 85 90 95

Leu Arg Phe Ser Leu Arg Asn His Asp Glu Ala Glu Ala Leu Ala Gln
 100 105 110

Ala Ala Asp Ile Leu Phe Leu Asp Val Trp Ala Ser Thr Pro Ala Phe
 115 120 125

Val Asp Ile Arg Leu Ala Glu Glu Val Thr Ala Tyr Thr Pro Leu Ile
 130 135 140

Asp Asn Leu Ala Glu Arg Ile Tyr Thr Thr Tyr Pro Ser Lys Lys Pro
 145 150 155 160

Ile Gly Leu Glu Gly Gln Ser Gly Phe Ala Ser Ser Ser Arg Pro Ala
 165 170 175

Pro Lys Phe Gly Asp Leu Phe Phe His Glu Tyr Gln Pro Leu Ser Val
 180 185 190

Ile Ile Pro Trp Met Arg Leu Leu Ala Ser Met Phe Pro Ser His Val

195	200	205
Arg Met Ile Ser Val Gly	Val Ser Tyr Glu Gly	Arg Glu Ile Pro Ala
210	215	220
Leu Arg Leu Ser Ala Gly	Ser Ser Thr Ala Ala	Ser Gly Pro Arg Lys
225	230	235
Thr Ile Ile Val Thr Gly	Gly Ser His Ala Arg	Glu Trp Ile Gly Thr
	245	250
Ser Thr Val Asn His Val	Met Tyr Thr Leu Ile	Thr Lys Tyr Gly Lys
	260	265
Ser Lys Ala Val Thr Arg	Leu Leu Gln Asp Phe	Asp Trp Ile Met Ile
	275	280
Pro Thr Ile Asn Pro Asp	Gly Tyr Val Tyr Thr	Trp Glu Thr Asp Arg
	290	295
Leu Trp Arg Lys Asn Arg	Gln Arg Thr Ser Leu	Arg Phe Cys Pro Gly
305	310	315
Ile Asp Leu Asp Arg Ala	Trp Gly Phe Glu Trp	Asp Gly Gly Arg Thr
	325	330
Arg Ala Asn Pro Cys Ser	Glu Asn Tyr Ala Gly	Asp Glu Pro Phe Glu
	340	345
Gly Met Glu Ala Gln Gln	Leu Ala Gln Trp Ala	Leu Asn Glu Thr Gln
	355	360
Asn Asn Asn Ala Asp Ile	Val Ser Phe Leu Asp	Leu His Ser Tyr Ser
	370	375
Gln Thr Ile Leu Tyr Pro	Phe Ser Tyr Ser Cys	Ser Ser Ile Pro Pro
385	390	395
Thr Leu Glu Ser Leu Glu	Glu Leu Gly Leu Gly	Leu Ala Lys Ala Ile
	405	410
Arg Tyr Ala Thr His Glu	Ile Tyr Asp Val Thr	Ser Ala Cys Glu Gly
	420	425
		430

Ile Val Thr Ala Ser Ala Ala Asp Asn Asn Pro Gly Arg Phe Phe Pro
 435 440 445

Ile Gly Gly Asn Ser Gly Gly Ser Ala Leu Asp Trp Phe Tyr His Gln
 450 455 460

Val His Ala Thr Tyr Ser Tyr Gln Ile Lys Leu Arg Asp Arg Gly Ser
 465 470 475 480

Tyr Gly Phe Leu Leu Pro Ser Glu His Ile Ile Pro Thr Gly Lys Glu
 485 490 495

Ile Tyr Asn Val Val Leu Lys Leu Gly Ser Phe Leu Ile Gly Gly Asp
 500 505 510

Ser Phe Asp Val Asp Trp Glu Ser Glu Leu Phe Asp Leu Ser Lys Asp
 515 520 525

Glu Ser Asp Leu Asp Ser Arg Tyr Ser Lys Ser Asn Asp Arg Ser Pro
 530 535 540

Ala Tyr Leu His Asn Ala Asn Gly Pro Leu Pro Asn Ile Asp Glu Asp
 545 550 555 560

Glu Asp Lys Glu Trp Val Met Val Glu Glu Glu Asp Tyr Thr Asp Asp
 565 570 575

Asp Asp Asp Asp Asp Asp Asp Asp Glu Glu Glu Glu Glu Glu Glu
 580 585 590

Asp Thr Tyr Trp Ala Thr Glu His Thr Tyr Glu Phe Arg Arg Arg Arg
 595 600 605

<210> 144

<211> 416

<212> PRT

<213> Aspergillus niger

<400> 144

Met Ala Phe Leu Lys Arg Ile Leu Pro Leu Leu Ala Leu Ile Leu Pro
 1 5 10 15

Ala Val Phe Ser Ala Thr Glu Gln Val Pro His Pro Thr Ile Gln Thr
 20 25 30

Ile Pro Gly Lys Tyr Ile Val Thr Phe Lys Ser Gly Ile Asp Asn Ala
 35 40 45

Lys Ile Glu Ser His Ala Ala Trp Val Thr Glu Leu His Arg Arg Ser
 50 55 60

Leu Glu Gly Arg Ser Thr Thr Glu Asp Asp Leu Pro Ala Gly Ile Glu
 65 70 75 80

Arg Thr Tyr Arg Ile Ala Asn Phe Ala Gly Tyr Ala Gly Ser Phe Asp
 85 90 95

Glu Lys Thr Ile Glu Glu Ile Arg Lys His Asp His Val Ala Tyr Val
 100 105 110

Glu Gln Asp Gln Val Trp Tyr Leu Asp Thr Leu Val Thr Glu Arg Arg
 115 120 125

Ala Pro Trp Gly Leu Gly Ser Ile Ser His Arg Gly Gly Ser Ser Thr
 130 135 140

Asp Tyr Ile Tyr Asp Asp Ser Ala Gly Glu Gly Thr Tyr Ala Tyr Val
 145 150 155 160

Val Asp Thr Gly Ile Leu Ala Thr His Asn Glu Phe Gly Gly Arg Ala
 165 170 175

Ser Leu Ala Tyr Asn Ala Ala Gly Gly Glu His Val Asp Asp Val Gly
 180 185 190

His Gly Thr His Val Ala Gly Thr Ile Gly Gly Lys Thr Tyr Gly Val
 195 200 205

Ser Lys Asn Ala His Leu Leu Ser Val Lys Val Phe Val Gly Glu Ser
 210 215 220

Ser Ser Thr Ser Val Ile Leu Asp Gly Phe Asn Trp Ala Ala Asn Asp
 225 230 235 240

Ile Val Ser Lys Asn Arg Thr Ser Lys Ala Ala Ile Asn Met Ser Leu

245 250 255
 Gly Gly Gly Tyr Ser Tyr Ala Phe Asn Asn Ala Val Glu Asn Ala Phe
 260 265 270
 Asp Glu Gly Val Leu Ser Cys Val Ala Ala Gly Asn Glu Asn Arg Asp
 275 280 285
 Ala Ala Arg Thr Ser Pro Ala Ser Ala Pro Asp Ala Ile Thr Val Ala
 290 295 300
 Ala Ile Asn Arg Ser Asn Ala Arg Ala Ser Phe Ser Asn Tyr Gly Ser
 305 310 315 320
 Val Val Asp Ile Phe Ala Pro Gly Glu Gln Val Leu Ser Ala Trp Thr
 325 330 335
 Gly Ser Asn Ser Ala Thr Asn Thr Ile Ser Gly Thr Ser Met Ala Thr
 340 345 350
 Pro His Val Thr Gly Leu Ile Leu Tyr Leu Met Gly Leu Arg Asp Leu
 355 360 365
 Ala Thr Pro Ala Ala Ala Thr Thr Glu Leu Lys Arg Leu Ala Thr Arg
 370 375 380
 Asn Ala Val Thr Asn Val Ala Gly Ser Pro Asn Leu Leu Ala Tyr Asn
 385 390 395 400
 Gly Asn Ser Gly Val Ser Lys Gly Gly Ser Asp Asp Gly Asp Glu Asp
 405 410 415

 <210> 145
 <211> 455
 <212> PRT
 <213> Aspergillus niger

 <400> 145

 Met Ile Thr Leu Leu Ser Ala Leu Phe Gly Ser Val Val Tyr Ala Ala
 1 5 10 15

 Thr Gln Thr Val Leu Gly Pro Glu Gly Ala Asp Pro Phe Thr Val Phe
 20 25 30

Arg Ser Pro His Ser Pro Ala Phe Ser Ile Arg Ile Gln Glu Gln Asn
 35 40 45
 Asp Ser Ile Cys Asp Ala Arg Ser Pro Gln Phe Thr Gly Trp Leu Asp
 50 55 60
 Ile Gly Pro Lys His Leu Phe Phe Trp Tyr Phe Glu Ser Gln Asn Asp
 65 70 75 80
 Pro Phe His Asp Pro Leu Thr Leu Trp Met Thr Gly Gly Pro Gly Asp
 85 90 95
 Ser Ser Met Ile Gly Leu Phe Glu Glu Val Gly Pro Cys Arg Ile Asn
 100 105 110
 Glu Phe Gly Asn Gly Thr Asp His Asn Pro Trp Ala Trp Thr Lys Asn
 115 120 125
 Ser Ser Leu Leu Phe Val Asp Gln Pro Val Asp Val Gly Phe Ser Tyr
 130 135 140
 Ile Asp Glu Gly Tyr Glu Leu Pro His Asp Ser Arg Glu Ala Ala Val
 145 150 155 160
 Asp Met His Arg Phe Leu Arg Leu Phe Ile Ser Glu Ile Phe Pro His
 165 170 175
 Lys Gln Phe Leu Pro Val His Leu Ser Gly Glu Ser Tyr Ala Gly Arg
 180 185 190
 Tyr Ile Pro Tyr Leu Ala Thr Gln Ile Leu Glu Gln Asn Glu Leu Tyr
 195 200 205
 Lys Asp Ser Pro Arg Ile Pro Leu Lys Ser Cys Leu Val Gly Asn Gly
 210 215 220
 Phe Met Ser Pro Lys Asp Ala Thr Phe Gly Tyr Trp Glu Thr Leu Cys
 225 230 235 240
 Thr Thr Asn Ser Gly Val Pro Ser Pro Ile Phe Asn Glu Thr Arg Cys
 245 250 255

Asp Ile Met Ala Ala Asn Met Pro His Cys Met Asp Leu Tyr Asp Ile
 260 265 270

Cys Ile Gln His Ser Asp Pro Ala Ile Cys His Ala Ala Gln Ser Val
 275 280 285

Cys Tyr Asp Ser Val Val Gly Leu Met Ala Lys Leu Leu Leu Arg Met
 290 295 300

Thr Thr Val Thr Ala Pro Cys Glu Ile Asp Glu Met Cys Tyr Ile Glu
 305 310 315 320

Ala Ala Leu Ile Glu Arg Tyr Leu Asn Ser Pro Ser Val Trp Glu Ala
 325 330 335

Leu Ser Pro Pro Gln Gln Val Thr Glu Tyr Lys Phe Val Ala Thr Ser
 340 345 350

Val Ile Asp Ala Phe Ala Gln Ser Ala Asp Gly Met Val Ser Ser Ser
 355 360 365

Lys Gln Ile Ala Phe Leu Leu Ala Asn Asn Val Asp Phe Leu Ala Tyr
 370 375 380

Gln Gly Asn Leu Asp Leu Ala Cys Asn Thr Ala Gly Asn Leu Arg Trp
 385 390 395 400

Ala Asn Ser Leu Ser Trp Lys Gly Gln Thr Glu Phe Thr Ala Lys Pro
 405 410 415

Leu Leu Pro Trp Glu Ile Gln Val Ser Val Gly Glu Gly Thr Asp Glu
 420 425 430

Thr Ser Arg Phe Ala Phe Val Thr Val Asp Asn Ala Gly His Leu Leu
 435 440 445

Arg Asp Ser Lys Ile Ser Asn
 450 455

<210> 146

<211> 791

<212> PRT

<213> Aspergillus niger

<400> 146

Met Arg Phe Leu Thr Tyr Ser Leu Pro Phe Ile Ala Ser Ala Ile Ser
 1 5 10 15

Leu Phe Gly Val Asn Val Gln Ala Arg Ser Gln Ala Pro Ser Ala Ile
 20 25 30

Arg His Val Ser Thr Leu Asp Gln Pro Thr Ile Lys Thr Pro Ser Gln
 35 40 45

Arg Val Asp His Leu Asp His Phe Asp Ile Thr Phe Asn Ile His Asp
 50 55 60

Lys His Gln Arg Ile Lys Leu Glu Leu Glu Pro Asn His Asp Ile Leu
 65 70 75 80

Ala Glu Asp Ala Ser Val Gln Tyr Leu Asp Ala Asp Gly Asn Val Arg
 85 90 95

Arg His Glu Pro Ile Ala Pro His Glu His Lys Val Phe Lys Gly Arg
 100 105 110

Ser Leu Leu Gly Arg Gly Lys Gly Met Trp Asp Pro Val Gly Trp Ala
 115 120 125

Arg Ile Tyr Leu Lys Gln Asp Gly Ser Glu Pro Leu Phe Glu Gly Val
 130 135 140

Phe Ser Ile Asp Gly Asp Asn His His Val Gln Leu Lys Ser Ala Tyr
 145 150 155 160

Met Glu Lys Lys Arg Pro Val Asp Val Asp Leu Pro Asp Ser Ala Thr
 165 170 175

Asp Tyr Met Ile Phe Tyr Arg Asp Ser Asp Met Val Arg Leu His Thr
 180 185 190

Glu Leu Lys Arg Ser Ser Leu Gly Ser Thr Ser Cys Gln Ala Asp Gln
 195 200 205

Leu Gly Phe Asn Thr Asn Pro Asn His Pro Val Leu Gln Pro Tyr Gly
 210 215 220

Gln Ala Glu Thr Asp Thr Trp Gly Ala Ile Ser Leu Asn Ser Leu Phe
 225 230 235 240

Gly Leu Asn Lys Arg Gln Ser Asp Ile Gly Ser Val Ser Gly Asn Ala
 245 250 255

Gly Gly Val Asn Leu Ala Ser Thr Ile Gly Asp Thr Ser Gly Cys Pro
 260 265 270

Ser Thr Lys Gln Val Ala Leu Ile Gly Val Ala Thr Asp Cys Ala Phe
 275 280 285

Thr Gly Ser Phe Asn Asn Glu Thr Ala Ala Lys Glu Trp Val Ile Ser
 290 295 300

Thr Val Asn Ser Ala Ser Asn Val Tyr Glu Lys Ser Phe Asn Ile Thr
 305 310 315 320

Ile Gly Leu Arg Asn Leu Thr Ile Thr Asp Ser Ser Cys Pro Asp Asn
 325 330 335

Pro Pro Ala Ala Thr Ala Trp Asn Met Pro Cys Ser Ser Gly Asn Leu
 340 345 350

Thr Ser Arg Leu Asp Leu Phe Ser Lys Trp Arg Gly Glu Gln Ser Asp
 355 360 365

Asp Asn Ala Tyr Trp Thr Leu Met Ser Asp Cys Ala Thr Gly Asn Glu
 370 375 380

Val Gly Leu Ser Trp Leu Gly Gln Leu Cys Asn Ser Asp Ala Ser Ser
 385 390 395 400

Asp Gly Ser Ser Thr Val Ser Gly Thr Asn Val Val Val Arg Ser Ser
 405 410 415

Gly Ser Asp Trp Gln Ile Phe Ala His Glu Ser Gly His Thr Phe Gly
 420 425 430

Ala Val His Asp Cys Asp Ser Gln Thr Cys Ala Glu Asp Leu Glu Ala
 435 440 445

Ser Ser Gln Cys Cys Pro Leu Thr Ser Ser Thr Cys Asn Ala Asn Gly
 450 455 460

Lys Tyr Ile Met Asn Pro Thr Thr Gly Thr Asp Ile Thr Ala Phe Ser
 465 470 475 480

Gln Cys Thr Ile Gly Asn Ile Cys Ala Ala Leu Gly Arg Asn Ser Val
 485 490 495

Lys Ser Ser Cys Leu Ser Ala Asn Arg Asp Val Thr Thr Tyr Thr Gly
 500 505 510

Ser Gln Cys Gly Asn Gly Ile Val Glu Ser Gly Glu Asp Cys Asp Cys
 515 520 525

Gly Gly Glu Asp Gly Cys Gly Asp Asn Asn Cys Cys Asp Ala Lys Thr
 530 535 540

Cys Lys Phe Lys Ser Gly Ala Val Cys Asp Asp Ser Asn Asp Ser Cys
 545 550 555 560

Cys Ser Ser Cys Gln Phe Ser Ser Ala Gly Thr Val Cys Arg Ala Ser
 565 570 575

Arg Gly Asp Cys Asp Val Ala Glu Thr Cys Ser Gly Asn Ser Ser Thr
 580 585 590

Cys Pro Thr Asp Ser Phe Lys Lys Asp Gly Thr Ser Cys Gly Ser Ser
 595 600 605

Gly Ser Gly Leu Ala Cys Ala Ser Gly Gln Cys Thr Ser Arg Asp Tyr
 610 615 620

Gln Cys Arg Ser Val Met Gly Ser Leu Leu His Ser Asn Asp Thr Tyr
 625 630 635 640

Ala Cys Ser Ser Phe Ser Ser Ser Cys Glu Leu Val Cys Thr Ser Pro
 645 650 655

Lys Ile Gly Thr Cys Tyr Ser Val Asn Gln Asn Phe Leu Asp Gly Thr
 660 665 670

Pro Cys Gly Ser Gly Gly Tyr Cys Ser Asn Gly Asp Cys Lys Gly Gln

675 680 685
 Asn Val Glu Ser Trp Ile Lys Asn His Lys Gly Ile Val Ile Gly Val
 690 695 700
 Ala Cys Ala Val Gly Ala Leu Ile Leu Leu Ala Leu Met Thr Cys Ile
 705 710 715 720
 Val Asn Arg Cys Arg Arg Ala Arg Ala Pro Lys Pro Val Pro Arg Pro
 725 730 735
 Val Pro Tyr Gly Pro Trp Pro Gly Ala Arg Pro Pro Pro Pro Pro Pro
 740 745 750
 Met Asn Gln Trp Pro Ala Arg Gly Tyr Gln Gly Leu Gly Asn Glu Pro
 755 760 765
 Pro Pro Pro Tyr Pro Gly Val Pro Gly Gln Pro Val Pro Gln His Met
 770 775 780
 Pro Pro Gln Gly Arg Tyr Ala
 785 790
 <210> 147
 <211> 481
 <212> PRT
 <213> Aspergillus niger
 <400> 147
 Met Arg Phe Leu Ser Ser Ala Ala Leu Phe Gly Leu Ala Tyr Ala Ser
 1 5 10 15
 Thr Gln Ala Val Leu Gln Pro Glu Glu Pro Ser Asp Phe Arg Thr Phe
 20 25 30
 His Ser Pro Tyr Ser Pro His His Ser Ile Arg Ile Arg Gln Gln Asn
 35 40 45
 Glu Ser Ile Cys Ala Ala His Ser Ala Gln Tyr Thr Gly Trp Leu Asp
 50 55 60
 Ile Gly Arg Lys His Leu Phe Phe Trp Tyr Phe Glu Ser Gln Asn Asp
 65 70 75 80

Pro Ala Asn Asp Pro Leu Thr Leu Trp Met Thr Gly Gly Pro Gly Gly
 85 90 95

Ser Ser Met Ile Gly Leu Phe Glu Glu Val Gly Pro Cys Leu Ile Asn
 100 105 110

Glu Tyr Gly Asn Gly Thr Tyr Tyr Asn Pro Trp Gly Trp Ser Arg Asn
 115 120 125

Ser Ser Leu Leu Phe Val Asp Gln Pro Val Asp Val Gly Phe Ser Tyr
 130 135 140

Val Asp Glu Gly Glu Asp Leu Pro Gly Asp Ser His Gln Ala Ala Ile
 145 150 155 160

Asp Met His Arg Phe Leu Gln Leu Phe Val Ser Glu Val Phe Pro Gln
 165 170 175

Leu Gln Thr Leu Pro Val His Leu Ser Gly Glu Ser Tyr Ala Gly His
 180 185 190

Tyr Val Pro Tyr Leu Gly Ser Gln Ile Val Gln Gln Asn Lys Leu Tyr
 195 200 205

Pro Thr Glu Pro Gln Val Leu Leu His Ser Cys Leu Val Gly Asn Gly
 210 215 220

Tyr Tyr Ser Pro Arg Asp Thr Thr Tyr Gly Tyr Trp Glu Thr Leu Cys
 225 230 235 240

Thr Thr Asn Pro Gly Val Pro Glu Pro Val Phe Asn Arg Thr Arg Cys
 245 250 255

Asp Ile Met Ala Ala Asn Met Pro Arg Cys Met Glu Val Ser Asp Val
 260 265 270

Cys Val Arg Asn Pro Asp Pro Ala Ile Cys His Ala Ala Ser Glu Val
 275 280 285

Cys Tyr Glu Gly Val Ile Gly Trp Tyr Asp Asp Glu Ser Gly Glu Gly
 290 295 300

Gly Arg Asn Arg Phe Asp Ile Thr Ala Pro Cys Ala Leu Asp Gly Ile
 305 310 315 320

Cys Tyr Ile Glu Ala Ala Arg Ile Glu Gln Tyr Leu Asn Thr Pro Ala
 325 330 335

Val Trp Ala Ala Leu Ser Pro Pro Lys Glu Ile Lys Glu Tyr Lys Val
 340 345 350

Thr Ser Asp Asn Val Ser Arg Ala Phe Asp Leu Thr Ser Asp Thr Met
 355 360 365

Thr Pro Ala Ser Glu Gln Val Ala Phe Leu Leu Ala Asn Gln Val His
 370 375 380

Phe Leu Ala Tyr Gln Gly Asn Leu Asp Leu Ala Cys Asn Thr Ala Gly
 385 390 395 400

Asn Leu Arg Trp Ala His Ser Leu Pro Trp Arg Gly Gln Val Glu Phe
 405 410 415

Ala Ser Lys Ala Leu Arg Pro Trp Ser Trp Val Asp Val Val Ser Gly
 420 425 430

Lys Gly Gly Val Ala Gly Thr Thr Lys Glu Glu Ser Arg Phe Ala Leu
 435 440 445

Val Thr Val Asp Gly Ala Gly His Phe Leu Pro Gln Asp Arg Pro Asp
 450 455 460

Ile Ala Leu Asp Met Met Val Arg Trp Ile Ser Gly Ala Ser Phe Thr
 465 470 475 480

Glu

<210> 148

<211> 319

<212> PRT

<213> Aspergillus niger

<400> 148

Met Thr Leu Leu Leu Asn Phe His Ala Leu Phe Thr Val Ile Leu Val
 1 5 10 15

Ala Asn Leu Ser Thr Arg Cys Ser Ala Leu Leu Ser Gly Arg Asp Phe
 20 25 30

Cys Ser Thr Pro Ala Pro Gly Glu Ser Leu Arg Ala Glu His Arg Arg
 35 40 45

Leu Tyr Asp Val Gln Ala Gln Arg Asp Ser Thr Ala Glu Glu Ser Arg
 50 55 60

Glu Val Val Pro Trp Ile Glu Ile Glu Thr Trp Phe His Ile Val Ser
 65 70 75 80

Ser Asn Glu Ala Ala Asn Thr Val Ser Asp Asp Met Ile Thr Ser Gln
 85 90 95

Leu Ser Tyr Leu Gln Lys Ala Tyr Glu Ser Ala Thr Ile Thr Tyr Arg
 100 105 110

Leu Glu Gly Ile Thr Arg His Ile Asn Asp Ser Trp Ala Arg Asn Asp
 115 120 125

Asp Glu Leu Gly Met Lys Asn Ala Leu Arg Arg Gly Asn Tyr Gly Thr
 130 135 140

Leu Asn Val Tyr Phe Gln Thr Asp Leu Gln Ala Ser Ser Asp Glu Asn
 145 150 155 160

Ser Arg Asp Tyr Pro Asn Asp Gly Asn Arg Arg Thr Asp Val Ser Asp
 165 170 175

Gln Ser Ser Ser Thr Val Leu Gly Phe Cys Thr Leu Pro Asp Pro Ser
 180 185 190

Val Asn Ser Ser Ser Pro Arg Ser Ser Tyr Ile Lys Asp Gly Cys Asn
 195 200 205

Val Leu Ala Asp Ile Met Pro Gly Gly Ser Leu Ala Gln Tyr Asn Lys
 210 215 220

Gly Gly Thr Ala Val His Glu Val Gly His Trp Asn Gly Leu Leu His
 225 230 235 240

Thr Phe Glu Gly Glu Ser Cys Ser Pro Asp Asn Glu Gly Asp Tyr Ile
 245 250 255

Asp Asp Thr Pro Glu Gln Ser Glu Pro Thr Ser Gly Cys Pro Ala Glu
 260 265 270

Lys Asp Ser Cys Pro Asp Leu Pro Gly Leu Asp Ala Ile His Asn Phe
 275 280 285

Met Asp Tyr Ser Ser Asp Asp Cys Tyr Glu Ser Phe Thr Pro Asp Gln
 290 295 300

Ala Glu Arg Met Arg Ser Met Trp Ser Ala Met Arg Glu Gly Lys
 305 310 315

<210> 149
 <211> 639
 <212> PRT
 <213> Aspergillus niger

<400> 149

Met His Val Ser Leu Phe Leu Leu Ser Val Thr Ala Ala Phe Ala Ser
 1 5 10 15

Pro Thr Pro His Asn Tyr Val Val His Glu Arg Arg Asp Ala Leu Pro
 20 25 30

Ser Val Trp Val Glu Glu Ser Arg Leu Asp Lys Gly Ala Leu Leu Pro
 35 40 45

Met Arg Ile Gly Leu Thr Gln Ser Asn Leu Asp Arg Gly His Asp Leu
 50 55 60

Leu Met Glu Val Ser His Pro Gln Ser Ser Arg Tyr Gly Lys His Leu
 65 70 75 80

Ser Ser Glu Glu Val His Asp Leu Phe Ala Pro Ser Asn Glu Ala Val
 85 90 95

Glu Thr Val Arg Thr Trp Ile Glu Ser Ala Gly Ile Ala Pro Ser Arg
 100 105 110

Ile Ser Gln Ser Tyr Asn Lys Gln Trp Leu Gln Phe Asp Ala His Ala

115	120	125
Ser Glu Val Glu Gln Leu Leu Gln Thr Glu Tyr Tyr Ile Tyr Thr His		
130	135	140
Ala Asp Thr Gly Ser Ser His Val Thr Cys His Glu Tyr His Val Pro		
145	150	155 160
Glu Thr Ile Gln Ser His Ile Asp Tyr Ile Thr Pro Gly Val Lys Met		
	165	170 175
Leu Glu Val Arg Gly Thr Pro Ser Lys Lys Arg Asp Ala Glu Lys Arg		
	180	185 190
Ser Leu Gly Ser Leu Pro Pro Ile Leu Ala Pro Leu Pro Ile Asn Ile		
	195	200 205
Thr Lys Ile Phe Asp Asp Pro Leu Ala His Cys Asp Leu Ala Val Thr		
	210	215 220
Pro Asp Cys Ile Arg Ala Met Tyr Asn Ile Thr Lys Gly Thr Thr Ala		
225	230	235 240
Thr Lys Gly Asn Glu Leu Gly Ile Phe Glu Asp Leu Gly Asp Ile Tyr		
	245	250 255
Ser Gln Asp Asp Leu Asn Leu Phe Phe Ala Asn Phe Ala Ser Asp Ile		
	260	265 270
Pro Gln Gly Thr His Pro Thr Leu Asp Ser Ile Asp Gly Ala Thr Ala		
	275	280 285
Pro Thr Asp Val Thr Asn Ala Gly Pro Glu Ser Asp Leu Asp Phe Gln		
	290	295 300
Ile Ala Tyr Pro Ile Ile Trp Pro Gln Asn Thr Ile Leu Tyr Gln Thr		
305	310	315 320
Asp Asp Pro Asn Tyr Glu Asp Asn Tyr Asn Phe Lys Gly Leu Leu Asn		
	325	330 335
Asn Phe Leu Tyr Ala Ile Asp Gly Ser Tyr Cys Asn Glu Thr Ser Ser		
	340	345 350

Leu Asp Pro Gln Tyr Pro Asp Pro Ser Pro Gly Gly Tyr Ser Ser Pro
 355 360 365

Lys Gln Cys Gly Val Tyr Thr Pro Thr Asn Val Ile Ser Ile Ser Tyr
 370 375 380

Gly Ser Pro Glu Ala Asp Leu Pro Ile Ala Tyr Gln Arg Arg Gln Cys
 385 390 395 400

His Glu Phe Met Lys Leu Gly Leu Gln Gly Ile Ser Val Val Val Ala
 405 410 415

Ser Gly Asp Ser Gly Val Ala Ser Ser Thr Gly Thr Cys Phe Gly Asp
 420 425 430

Ala Asp Asn Val Phe Val Pro Asp Phe Pro Ala Thr Cys Pro Tyr Leu
 435 440 445

Thr Ala Val Gly Gly Thr Tyr Leu Pro Leu Gly Ala Asp Ala Ala Lys
 450 455 460

Asp Gln Glu Ile Ala Val Thr Arg Phe Pro Ser Gly Gly Gly Phe Ser
 465 470 475 480

Asn Ile Tyr Ala Arg Pro Ser Tyr Gln Asn His Ser Val Glu Thr Tyr
 485 490 495

Phe Ser Thr Thr Ser Asp Asp Leu Thr Tyr Pro Tyr Tyr Ser Gly Val
 500 505 510

Asn Tyr Thr Asp Phe Ser Asn Thr Asp Gly Val Tyr Asn Arg Ile Gly
 515 520 525

Arg Gly Tyr Pro Asp Val Ser Ala Ile Ala Asp Asn Ile Ile Ile Tyr
 530 535 540

Asn Gln Gly Glu Ala Thr Leu Val Gly Gly Thr Ser Ala Ala Ala Pro
 545 550 555 560

Ala Phe Ala Ala Met Leu Thr Arg Ile Asn Glu Glu Arg Leu Ala Lys
 565 570 575

Gly Lys Ser Thr Val Gly Phe Val Asn Pro Val Leu Tyr Glu His Pro
 580 585 590

Glu Ala Phe Arg Asp Val Thr Val Gly Ser Asn Pro Gly Cys Gly Thr
 595 600 605

Asp Gly Phe Pro Val Ala Gly Gly Trp Asp Pro Val Thr Gly Leu Gly
 610 615 620

Thr Pro Arg Phe Glu Asp Leu Met Asp Ile Phe Val Gly Asp Asp
 625 630 635

<210> 150
 <211> 371
 <212> PRT
 <213> Aspergillus niger

<400> 150

Met Ala Ser Lys Thr Leu Leu Leu Ile Pro Ala Leu Ala Thr Ala Ala
 1 5 10 15

Leu Gly Ser Val Leu Asp Leu Asp Ile Lys Val Asp Leu Gly Thr Pro
 20 25 30

Gly Gly Pro Phe Asp Leu Met Tyr Asp Thr Gly Ser Ser Thr Leu Trp
 35 40 45

Val Leu Asp Ser Asn Cys Thr Asp Asp Cys Pro Asn Val Ser Gly Tyr
 50 55 60

Ser Arg His Gly Tyr Asn Leu Thr Ser Thr Gly Val Asn Leu Gly Val
 65 70 75 80

Asn Asp Ser Ile Ala Tyr Ser Gly Gly Thr Val Ser Gly Phe Thr Ala
 85 90 95

Thr Asp Ile Leu Thr Val Pro Asp Thr Asn Val Ser Tyr Arg Gln Ser
 100 105 110

Phe Ala Val Ile Thr Asp Ser Thr Trp Ala Ala Leu Ala Ala Asp Gly
 115 120 125

Phe Ile Gly Leu Ala Ser Ser Thr Ile Ala Phe Lys Asn Thr Thr Thr

130	135	140
Ala Val Glu Gln Met Met Gln Asp Gly Leu Leu Asp Glu Pro Arg Phe		
145	150	155 160
Ala Ile Tyr Ala Gly Ser Gly Glu Ser Thr Val Thr Asn Pro Asn Pro		
	165	170 175
Glu Asn Asn Gly Val Phe Thr Phe Gly Gly Ser His Glu Glu Thr Tyr		
	180	185 190
Ala Asp Gly Glu Leu Gln Trp Met Lys Met Leu Ser Pro Phe Glu Ile		
	195	200 205
Tyr Lys Thr Asn Leu Leu Gly Ile Gln Gly His Asn Asn Ser Asp Gly		
	210	215 220
Gln Ala Leu Ser Ser Asp Val Leu Asn Trp Tyr Gly Gln Thr Asn Leu		
225	230	235 240
Phe Asn Val Ala Gly Ala Ser Ser Ile Ser Ile Pro Asn Asp Gln Ile		
	245	250 255
Glu Ala Met Tyr Ala Leu Thr Pro Phe Ser Tyr Ala Asp Ile Ser Ser		
	260	265 270
Gly Tyr Arg Pro Leu Cys Ser Asp Phe Asn Asp Thr Trp Ser Ile Ser		
	275	280 285
Phe Thr Met Gly Phe Tyr Gly Glu Gly Val Thr Phe Asn Leu Thr Gly		
	290	295 300
Asp Gln Leu Ala Val Pro Gly Tyr Gln Asp Asp Asp His Cys Phe Pro		
305	310	315 320
Pro Phe Asn Pro Trp Asp Ser Tyr Asn Thr Ile Ile Gly Gln His Trp		
	325	330 335
Leu Ser Asn Phe Tyr Ala Val Phe Asp Phe Gly Ser Phe Asp Pro Glu		
	340	345 350
Thr Tyr Asp Ile Arg Val Gly Leu Ala Pro Leu Lys Lys Glu Tyr Leu		
	355	360 365

Pro Ser Ala
370

<210> 151
<211> 414
<212> PRT
<213> *Aspergillus niger*

<400> 151

Met Phe Pro Cys Ser Arg Ile Trp Ser Leu Leu Val Ala Ala Ala Thr
1 5 10 15

Ala Ser Ala Val Pro Thr Ser Leu Ala Thr Thr His Leu Gln Ser Val
20 25 30

Asp Leu Leu Leu Thr Arg Ser Ser Tyr Gly Phe Leu Thr Asp Ile Ala
35 40 45

Leu Gly Thr Pro Gly Gln Ser Leu Pro Tyr Leu Val Asp Trp Thr Trp
50 55 60

Thr Gly His Tyr Val Val Thr Thr Leu Cys Tyr Asn Asp Pro Thr Ala
65 70 75 80

Thr Tyr Asp Cys Leu Asn Val Asp Gln Lys Ile Phe Asn Gln Thr Leu
85 90 95

Ser Ser Thr Phe Ile Asn Gln Thr Asp Gln Tyr Gly Tyr Leu Tyr Trp
100 105 110

Asp Pro Asn His Phe Tyr Phe Thr Glu Pro Ala Ala Ala Asp Val Ala
115 120 125

Thr Asp Met Leu Arg Ile Gly Pro Thr Ala Val Asn Thr Thr Ile Gln
130 135 140

Ala Ala Asn Phe Val Phe Asn Glu Thr Ile Ser Ala Phe Pro Phe Ser
145 150 155 160

Gly Val Tyr Gly Leu Ser Pro Val Phe Gln Gly Asp Asn Arg Ser Val
165 170 175

Gln Ala Ser Phe Tyr Gln Gly Trp Arg Ser Gly Ala Trp His Ser Pro
 180 185 190

Ile Val Ser Phe Ile Tyr Cys His Asp Asn Ala Thr Lys Ala Val Cys
 195 200 205

Ser Gly Tyr Asp Gly Leu Gln Thr Leu Gly Gly Tyr Asn Thr Ser His
 210 215 220

Val Gln Gly Asp Ile Thr Trp Tyr Asp Ile Ile Val Thr Glu Ala Ile
 225 230 235 240

Asn Thr Leu Asp Phe Val Tyr Ala Pro Ala Val Ile Asn Tyr Trp Ala
 245 250 255

Leu Asn Leu Thr Arg Phe Ser Ile Gly Asp Glu Glu Gln Glu Leu Asn
 260 265 270

Lys Thr Thr Thr Leu Asp Gly Lys Gln Ala Ala Val Ala Ala Phe Asp
 275 280 285

His Ala Ser Tyr Gly Arg Gly Ala Pro Val Ser Val Tyr Gly Tyr Gln
 290 295 300

Arg Leu Val Glu Leu Val Gly Ala Lys Ala Val Thr Leu Ser Asp Pro
 305 310 315 320

Pro Asn Asn Gly Glu Gln Gly Phe Tyr Gln Phe Asp Cys Arg Asn Ser
 325 330 335

Ser Leu Leu Pro Pro Leu Arg Tyr Glu Phe Ala Gly Ser Glu Arg Ala
 340 345 350

Trp Glu Ile Val Pro Glu Asn Tyr Val Glu Val Leu Ala Asn Gly Thr
 355 360 365

Asn Lys Cys Thr Phe Asn Val Arg Thr Leu Gly Asp Gly Ala Met Val
 370 375 380

Met Gly Asn Phe Gly Glu Thr Phe Ala Ile Asp Lys Tyr Val Met Phe
 385 390 395 400

Asp Phe Glu Lys Leu Gln Val Gly Ile Ala Asp Phe Ala Trp

405

410

<210> 152
 <211> 480
 <212> PRT
 <213> Aspergillus niger

<400> 152

Met His Leu Pro Gln Arg Leu Val Thr Ala Ala Cys Leu Cys Ala Ser
 1 5 10 15

Ala Thr Ala Phe Ile Pro Tyr Thr Ile Lys Leu Asp Thr Ser Asp Asp
 20 25 30

Ile Ser Ala Arg Asp Ser Leu Ala Arg Arg Phe Leu Pro Val Pro Lys
 35 40 45

Pro Ser Asp Ala Leu Ala Asp Asp Ser Thr Ser Ser Ala Ser Asp Glu
 50 55 60

Ser Leu Ser Leu Asn Ile Lys Arg Ile Pro Val Arg Arg Asp Asn Asp
 65 70 75 80

Phe Lys Ile Val Val Ala Glu Thr Pro Ser Trp Ser Asn Thr Ala Ala
 85 90 95

Leu Asp Gln Asp Gly Ser Asp Ile Ser Tyr Ile Ser Val Val Asn Ile
 100 105 110

Gly Ser Asp Glu Lys Ser Met Tyr Met Leu Leu Asp Thr Gly Gly Ser
 115 120 125

Asp Thr Trp Val Phe Gly Ser Asn Cys Thr Ser Thr Pro Cys Thr Met
 130 135 140

His Asn Thr Phe Gly Ser Asp Asp Ser Ser Thr Leu Glu Met Thr Ser
 145 150 155 160

Glu Glu Trp Ser Val Gly Tyr Gly Thr Gly Ser Val Ser Gly Leu Leu
 165 170 175

Gly Lys Asp Lys Leu Thr Ile Ala Asn Val Thr Val Arg Met Thr Phe
 180 185 190

Gly Leu Ala Ser Asn Ala Ser Asp Asn Phe Glu Ser Tyr Pro Met Asp
 195 200 205

Gly Ile Leu Gly Leu Gly Arg Thr Asn Asp Ser Ser Tyr Asp Asn Pro
 210 215 220

Thr Phe Met Asp Ala Val Ala Glu Ser Asn Val Phe Lys Ser Asn Ile
 225 230 235 240

Val Gly Phe Ala Leu Ser Arg Ser Pro Ala Lys Asp Gly Thr Val Ser
 245 250 255

Phe Gly Thr Thr Asp Lys Asp Lys Tyr Thr Gly Asp Ile Thr Tyr Thr
 260 265 270

Asp Thr Val Gly Ser Asp Ser Tyr Trp Arg Ile Pro Val Asp Asp Val
 275 280 285

Tyr Val Gly Gly Thr Ser Cys Asp Phe Ser Asn Lys Ser Ala Ile Ile
 290 295 300

Asp Thr Gly Thr Ser Tyr Ala Met Leu Pro Ser Ser Asp Ser Lys Thr
 305 310 315 320

Leu His Ser Leu Ile Pro Gly Ala Lys Ser Ser Gly Ser Tyr His Ile
 325 330 335

Ile Pro Cys Asn Thr Thr Thr Lys Leu Gln Val Ala Phe Ser Gly Val
 340 345 350

Asn Tyr Thr Ile Ser Pro Lys Asp Tyr Val Gly Ala Thr Ser Gly Ser
 355 360 365

Gly Cys Val Ser Asn Ile Ile Ser Tyr Asp Leu Phe Gly Asp Asp Ile
 370 375 380

Trp Leu Leu Gly Asp Thr Phe Leu Lys Asn Val Tyr Ala Val Phe Asp
 385 390 395 400

Tyr Asp Glu Leu Arg Val Gly Phe Ala Glu Arg Ser Ser Asn Thr Thr
 405 410 415

Ser Ala Ser Asn Ser Thr Ser Ser Gly Thr Ser Ser Thr Ser Gly Ser
 420 425 430

Thr Thr Thr Gly Ser Ser Thr Thr Thr Thr Ser Ser Ala Ser Ser Ser
 435 440 445

Ser Ser Ser Asp Ala Glu Ser Gly Ser Ser Met Thr Ile Pro Ala Pro
 450 455 460

Gln Tyr Phe Phe Ser Ala Leu Ala Ile Ala Ser Phe Met Leu Trp Leu
 465 470 475 480

<210> 153
 <211> 466
 <212> PRT
 <213> Aspergillus niger

<400> 153

Met Thr Ser Ser Thr Leu Arg Leu Ala Val Ala Leu Ala Leu Ser Thr
 1 5 10 15

Cys Ser Ser Ala Leu Ser Ser Gln Arg Asp Asp Ser Leu Val Val Pro
 20 25 30

Phe Pro Phe Gly Asn Leu Glu Asp Val His Ile Ala Lys Arg Asp Ser
 35 40 45

Ser Lys Thr Val Glu Ala Pro Leu Val Ile Tyr Gly Asp Ser Tyr Trp
 50 55 60

Met Asn Ala Ser Ile Gly Thr Pro Ala Gln Ser Leu Ser Phe Leu Leu
 65 70 75 80

Asp Leu Thr Arg Ser Arg Val Glu Pro Ala Tyr Thr Leu Asp Glu Asn
 85 90 95

Tyr Glu Cys Ser Asp Asp Glu Leu Cys Ser Glu Phe Gly Phe Tyr Lys
 100 105 110

Pro Thr Asp Ser Ser Thr Tyr Gln His Leu Thr Tyr Thr Gln Arg His
 115 120 125

Asp Ala Gly Val Asp Tyr Ser Tyr Leu Asp Thr Ile Thr Leu Gly Asp
 130 135 140

His Ala Thr Asp Asn Val Pro Leu Asp Met Tyr Leu Leu Ser Tyr Ile
 145 150 155 160

Ser Tyr Ser Ser Leu Gly Leu Ser Ser Val Asn Thr Ser Phe Pro Tyr
 165 170 175

Ile Leu Val Asp Arg Gly Leu Thr Thr Ser Pro Ser Phe Ser Leu Ile
 180 185 190

Gly Asp Asn Gly Asn Thr Thr Thr Pro Ser Ile Ile Phe Gly Gly Ile
 195 200 205

Asn Thr Ser Lys Phe Asn Gly Pro Leu Gln Ala Phe Ser Phe Ala Asp
 210 215 220

His Ser Ile Thr Asn Asn Pro Phe Val Thr Val Glu Ala Asp Ser Leu
 225 230 235 240

Gln Leu Thr Thr Asn Thr Asn Asp Asn Ser Thr Tyr Pro Ile Pro Ser
 245 250 255

Ser Thr Pro Met Met Leu Arg Thr Glu Glu Leu Ile Thr Tyr Leu Pro
 260 265 270

Asn Ser Thr Val Gln Ser Leu Tyr Thr Asp Leu Asn Ile Thr Met Asp
 275 280 285

Gly Val Ile Ser Thr Ser Arg Phe Tyr Gly Val Leu Pro Cys Ala Arg
 290 295 300

Gln Glu Thr Glu Ser His Thr Ile Ser Leu Ala Ile Gly Asn Met Thr
 305 310 315 320

Phe Ser Val Ser Trp Asp Glu Leu Phe Val Pro Trp Thr Arg Asp Gly
 325 330 335

Leu Cys Lys Phe Gly Ile Gln Ala Gln Asp Ser Asp Tyr Lys Thr Arg
 340 345 350

Ala Glu Leu Gly Val Pro Phe Leu Arg Arg Met Tyr Val Ala Val Asp
 355 360 365

Tyr Asn Asn Gln Phe Val Gly Val Ala Thr Leu Lys Asp Asp Asp Asp
 370 375 380

Gln Asn Gly Gly Glu Asp Glu Ile Val Glu Ile Gly Thr Gly Thr Ala
 385 390 395 400

Leu Pro Ser Ala Val Gly Asp Trp Pro Ala Ser Val Thr Ala Tyr Thr
 405 410 415

Pro Ala Ala Ser Thr Gly Thr Ala Ala Ala Thr Leu Thr Phe Thr Thr
 420 425 430

Ala Thr Ser Ser Gly Gly Gly Val Val Pro Thr Gly Leu Ser Glu Leu
 435 440 445

Gly Arg Ala Phe Leu Val Pro Gly Val Leu Gly Met Ala Val Leu Gln
 450 455 460

Ala Val
 465

<210> 154
 <211> 543
 <212> PRT
 <213> Aspergillus niger

<400> 154

Met Met Arg Pro Ile Leu Leu Pro Leu Leu Gly Val Phe Leu Gln Thr
 1 5 10 15

Ser Ser Ala Ser Asn Pro Tyr Val Met Ser Trp Ser Ser Gln Ala Tyr
 20 25 30

Gly Pro Asp Gly Pro Trp Gln Ala Val Ser Ile Asp Val Gly Ser Asn
 35 40 45

Gln Gln Thr Val Asp Leu Tyr Pro Gly Ala Asn Tyr Ala Ser Thr Ile
 50 55 60

Leu Met Ser Thr Leu Cys Thr Asn Lys Thr Leu Ser Ser Thr Cys Tyr
 65 70 75 80

Ala Ala Glu Ala Gly Thr Phe Asn Gln Asn Thr Ser Thr Thr Ala Tyr

85	90	95
Thr Thr Ala Ser Ser Trp Glu Thr	Thr Tyr Trp Ala Val Glu Gly Gly	
100	105	110
Ser Gln Glu Ala Val Leu Gly Asp Glu Val Thr Leu Gly Ser Phe Val		
115	120	125
Val Pro Asn Val Ser Phe Glu Ala Ile Tyr Gln Thr Tyr Gln Thr Tyr		
130	135	140
Pro Asn Gly Ile Ala Tyr Pro Val Ser Val Gly Ser Leu Ala Leu Gly		
145	150	155
Gly Pro Tyr Leu Ser Asp Thr Val Ser Asn Ser Thr Val Leu Asn Met		
165	170	175
Ile Ala Gly Trp Leu Tyr Ser Ser Asn Asp Ile Pro Ser Tyr Ser Tyr		
180	185	190
Gly Met His Ile Gly Ser Val Asp Pro Lys Ile Pro Gly Ser Leu Ile		
195	200	205
Leu Gly Gly Tyr Asp Lys Ser Arg Val Ile Gly Asp Val Ser Ala Gln		
210	215	220
Gly Val Val Ser Ser Ser Gly Leu Leu Glu Leu Glu Leu Lys Asp Ile		
225	230	235
Gly Leu Gly Val Ala Ala Gly Ser Ser Pro Phe Ser Phe Asn Asn Glu		
245	250	255
Ser Gly Leu Phe Leu Gln Ser Ser Gly Ser Val Gln Ala Lys Thr Val		
260	265	270
Gln Ile Asp Pro Thr Lys Pro Tyr Met Tyr Leu Pro Gln Ala Thr Cys		
275	280	285
Asp Ala Ile Thr Ser Thr Met Pro Ile Ser Phe Asn Ser Ser Leu Gly		
290	295	300
Leu Tyr Phe Trp Asp Thr Thr Ser Asp Asp Tyr Leu Asn Ile Thr Ser		
305	310	315
		320

Ser Ala Ala Tyr Leu Ser Phe Val Phe Asn Met Asn Gly Val Asn Asn
 325 330 335

Lys Asn Ile Thr Ile Lys Ile Pro Phe Ser Gln Leu Asn Leu Thr Leu
 340 345 350

Gln Glu Pro Leu Val Asp Gln Asn Val Thr Tyr Phe Pro Cys Phe Leu
 355 360 365

Thr Thr Ser Thr Pro Val Leu Gly Arg Ala Phe Leu Gln Ser Ala Phe
 370 375 380

Val Gly Val Asn Trp Phe Asn Gly Asn Asn Ser Gly Thr Trp Phe Leu
 385 390 395 400

Ala Gln Ala Pro Gly Pro Gly Tyr Ala Ser Glu Asp Ile Thr Arg Ile
 405 410 415

Ala Val Ser Asp Thr Ser Leu Ser Ala Ser Asn Gly Thr Trp Glu Glu
 420 425 430

Thr Trp Ala Thr Tyr Trp Gly Ile Lys Thr Ser Asp Asn Ser Ser Ser
 435 440 445

Ser Lys Ser Gly Leu Ser Ser Gly Ala Lys Ile Gly Ile Gly Val Gly
 450 455 460

Val Gly Val Gly Gly Ala Val Leu Ile Ala Ala Gly Ile Ala Ile Ala
 465 470 475 480

Phe Cys Leu Arg Arg Arg Arg Gly Ala Ser Gln Glu Ala Ala Gly Glu
 485 490 495

Gln Arg Arg Ser Met Phe Arg Gly Phe Ala Glu Leu Pro Gly Gly Ala
 500 505 510

His Ser Glu Pro Ala Lys Glu Leu Asp Thr Lys Met His Lys Pro Pro
 515 520 525

Gln Glu Met Met Ala Ser Gln Glu Val Glu Arg Tyr Glu Leu Gly
 530 535 540

<210> 155
 <211> 844
 <212> PRT
 <213> *Aspergillus niger*

<400> 155

Met Arg Leu Thr Gly Gly Val Ala Ala Ala Leu Gly Leu Cys Ala Ala
 1 5 10 15

Ala Ser Ala Ser Leu His Pro His Arg Ser Tyr Glu Thr His Asp Tyr
 20 25 30

Phe Ala Leu His Leu Asp Glu Ser Thr Ser Pro Ala Asp Val Ala Gln
 35 40 45

Arg Leu Gly Ala Arg His Glu Gly Pro Val Gly Glu Leu Pro Ser His
 50 55 60

His Thr Phe Ser Ile Pro Arg Glu Asn Ser Asp Asp Val His Ala Leu
 65 70 75 80

Leu Asp Gln Leu Arg Asp Arg Arg Arg Leu Arg Arg Arg Ser Gly Asp
 85 90 95

Asp Ala Ala Val Leu Pro Ser Leu Val Gly Arg Asp Glu Gly Leu Gly
 100 105 110

Gly Ile Leu Trp Ser Glu Lys Leu Ala Pro Gln Arg Lys Leu His Lys
 115 120 125

Arg Val Pro Pro Thr Gly Tyr Ala Ala Arg Ser Pro Val Asn Thr Gln
 130 135 140

Asn Asp Pro Gln Ala Leu Ala Ala Gln Lys Arg Ile Ala Ser Glu Leu
 145 150 155 160

Gly Ile Ala Asp Pro Ile Phe Gly Glu Gln Trp His Leu Tyr Asn Thr
 165 170 175

Val Gln Leu Gly His Asp Leu Asn Val Thr Gly Ile Trp Leu Glu Gly
 180 185 190

Val Thr Gly Gln Gly Val Thr Thr Ala Ile Val Asp Asp Gly Leu Asp

195	200	205
Met Tyr Ser Asn Asp Leu Arg Pro Asn Tyr Phe Ala Ala Gly Ser Tyr		
210	215	220
Asp Tyr Asn Asp Lys Val Pro Glu Pro Arg Pro Arg Leu Ser Asp Asp		
225	230	235 240
Arg His Gly Thr Arg Cys Ala Gly Glu Ile Gly Ala Ala Lys Asn Asp		
	245	250 255
Val Cys Gly Val Gly Val Ala Tyr Asp Ser Arg Ile Ala Gly Ile Arg		
	260	265 270
Ile Leu Ser Ala Pro Ile Asp Asp Thr Asp Glu Ala Ala Ala Ile Asn		
	275	280 285
Tyr Ala Tyr Gln Glu Asn Asp Ile Tyr Ser Cys Ser Trp Gly Pro Tyr		
	290	295 300
Asp Asp Gly Ala Thr Met Glu Ala Pro Gly Thr Leu Ile Lys Arg Ala		
305	310	315 320
Met Val Asn Gly Ile Gln Asn Gly Arg Gly Gly Lys Gly Ser Val Phe		
	325	330 335
Val Phe Ala Ala Gly Asn Gly Ala Ile His Asp Asp Asn Cys Asn Phe		
	340	345 350
Asp Gly Tyr Thr Asn Ser Ile Tyr Ser Ile Thr Val Gly Ala Ile Asp		
	355	360 365
Arg Glu Gly Asn His Pro Pro Tyr Ser Glu Ser Cys Ser Ala Gln Leu		
	370	375 380
Val Val Ala Tyr Ser Ser Gly Ala Ser Asp Ala Ile His Thr Thr Asp		
385	390	395 400
Val Gly Thr Asp Lys Cys Ser Thr Thr His Gly Gly Thr Ser Ala Ala		
	405	410 415
Gly Pro Leu Ala Ala Gly Thr Val Ala Leu Ala Leu Ser Val Arg Pro		
	420	425 430

Glu Leu Thr Trp Arg Asp Val Gln Tyr Leu Met Ile Glu Ala Ala Val
 435 440 445

Pro Val His Glu Asp Asp Gly Ser Trp Gln Asp Thr Lys Asn Gly Lys
 450 455 460

Lys Phe Ser His Asp Trp Gly Tyr Gly Lys Val Asp Thr Tyr Thr Leu
 465 470 475 480

Val Lys Arg Ala Glu Thr Trp Asp Leu Val Lys Pro Gln Ala Trp Leu
 485 490 495

His Ser Pro Trp Gln Arg Val Glu His Glu Ile Pro Gln Gly Glu Gln
 500 505 510

Gly Leu Ala Ser Ser Tyr Glu Val Thr Glu Asp Met Leu Lys Gly Ala
 515 520 525

Asn Leu Glu Arg Leu Glu His Val Thr Val Thr Met Asn Val Asn His
 530 535 540

Thr Arg Arg Gly Asp Leu Ser Val Glu Leu Arg Ser Pro Asp Gly Arg
 545 550 555 560

Val Ser His Leu Ser Thr Pro Arg Arg Pro Asp Asn Gln Glu Val Gly
 565 570 575

Tyr Val Asp Trp Thr Phe Met Ser Val Ala His Trp Gly Glu Ser Gly
 580 585 590

Ile Gly Lys Trp Thr Val Ile Val Lys Asp Thr Asn Val Asn Glu His
 595 600 605

Thr Gly Gln Phe Ile Asp Trp Arg Leu Asn Leu Trp Gly Glu Ala Ile
 610 615 620

Asp Gly Ala Glu Gln Pro Leu His Pro Met Pro Thr Glu His Asp Asp
 625 630 635 640

Asp His Ser Tyr Glu Glu Gly Asn Val Ala Thr Thr Ser Ile Ser Ala
 645 650 655

Val Pro Thr Lys Thr Glu Leu Pro Asp Lys Pro Thr Gly Gly Val Asp
660 665 670

Arg Pro Val Asn Val Lys Pro Thr Thr Ser Ala Met Pro Thr Gly Ser
675 680 685

Leu Thr Glu Pro Ile Asp Asp Glu Glu Leu Gln Lys Thr Pro Ser Thr
690 695 700

Glu Ala Ser Ser Thr Pro Ser Pro Ser Pro Thr Thr Ala Ser Asp Ser
705 710 715 720

Ile Leu Pro Ser Phe Phe Pro Thr Phe Gly Ala Ser Lys Arg Thr Gln
725 730 735

Val Trp Ile Tyr Ala Ala Ile Gly Ser Ile Ile Val Phe Cys Ile Gly
740 745 750

Leu Gly Val Tyr Phe His Val Gln Arg Arg Lys Arg Ile Arg Asp Asp
755 760 765

Ser Arg Asp Asp Tyr Asp Phe Glu Met Ile Glu Asp Glu Asp Glu Leu
770 775 780

Gln Ala Met Asn Gly Arg Ser Asn Arg Ser Arg Arg Arg Gly Gly Glu
785 790 795 800

Leu Tyr Asn Ala Phe Ala Gly Glu Ser Asp Glu Glu Pro Leu Phe Ser
805 810 815

Asp Glu Asp Asp Glu Pro Tyr Arg Asp Arg Gly Ile Ser Gly Glu Gln
820 825 830

Glu Arg Glu Gly Ala Asp Gly Glu His Ser Arg Arg
835 840

<210> 156

<211> 149

<212> PRT

<213> Aspergillus niger

<400> 156

Met Lys Thr Phe Ser Thr Val Thr Ser Leu Leu Ala Leu Phe Ser Ser

[illegible]

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<210> 157
<211> 296
<212> PRT
<213> Aspergillus niger
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<400> 157

Met Ala Gln Ile Phe Trp Leu Ser Leu Phe Leu Leu Val Ser Trp Val
1 5 10 15

Arg Ala Glu Ser Asn Arg Thr Glu Val Asp Leu Ile Phe Pro Arg Asn
20 25 30

Asp Thr Phe Ala Pro Met Pro Leu Met Pro Val Val Phe Ala Val Gln
35 40 45

Ala Pro Ser Val Ala His Lys Val Asn Thr Tyr Ile Glu Tyr Gly Tyr
 50 55 60

Tyr Pro Val Gly Arg Pro Asn Glu Thr Val Ile Gly Gln Thr Asp His
 65 70 75 80

Val Ser Asp Ser Thr Asn Glu Thr Thr Tyr Phe Ser Val Ser Gly Ile
 85 90 95

Gly Arg Thr Phe Asn Thr Thr Gly Ser Trp Glu Leu Phe Trp Arg Leu
 100 105 110

Arg Trp Thr Asn Cys Ser Ile Ser Glu Asp Ser Arg Tyr Tyr Asn Gln
 115 120 125

Ser Tyr Pro Trp Ile Ser Ser Pro Tyr Ile Asp Gly Ser Leu Asn Ile
 130 135 140

Asp Lys Val Tyr Glu Gly Phe His Tyr Thr Ala Tyr Asn Val Ile Val
 145 150 155 160

Asp Arg Val Thr Phe Ser Thr Arg Glu Asp Ala Ser Gln Pro Asn Leu
 165 170 175

Thr Thr Leu Thr Asn Ser Glu Asn Cys Asp Lys Val Ser Ser Leu Ala
 180 185 190

Leu Leu Ser Ile Val Asp Ser Leu Arg Ile Pro Pro Gln Leu Pro Gln
 195 200 205

Glu Asp Ile Asp Thr Val Ser Met Cys Pro Gln Leu Ala Asp Ala Arg
 210 215 220

Leu Asn Ser Thr Ser Thr Ser Ser Pro Cys Ser Val Ser Ile Ser Pro
 225 230 235 240

Glu Val Glu Ser Asn Ile Leu Ala Lys Ile Ala Asp Asn Glu Cys Asn
 245 250 255

Asn Ala Leu His Pro Ala Val Ser Cys Thr Thr Glu Glu Thr Lys Glu
 260 265 270

Gly Ser Ala Ser Ser His Asp His Gly His Ala Val Trp Leu Val Ile
 275 280 285

Thr Leu Ala Phe Ala Phe Leu Phe
 290 295

<210> 158
 <211> 310
 <212> PRT
 <213> *Aspergillus niger*

<400> 158

Met Gly Gly Arg Asp Val Ala Ile Leu Ser Arg His Phe Ala Val Thr
 1 5 10 15

Ser Ser Gln Ser Val Asn Gly Val Val Ser Gly Met Phe Gln His Thr
 20 25 30

Val Thr Ser Ser Pro Ser Phe Thr Thr Asn Gln Phe Phe Lys Lys Lys
 35 40 45

Phe Thr Ala Ala Ile Ala Thr Ala Ile Phe Ala Ser Val Ala Val Ala
 50 55 60

Ala Pro Gln Arg Gly Leu Glu Ala Arg Leu Lys Ala Arg Gly Ser Ser
 65 70 75 80

Lys Gly Ser Arg Pro Leu Gln Ala Val Ala Arg Pro Ala Ser Thr Lys
 85 90 95

Asn Gln Thr Asn Val Glu Tyr Ser Ser Asn Trp Ser Gly Ala Val Leu
 100 105 110

Val Glu Pro Pro Ser Ala Ala Ala Thr Tyr Thr Ala Val Thr Gly Thr
 115 120 125

Phe Thr Val Pro Glu Pro Thr Gly Asn Ser Gly Gly Ser Gln Ala Ala
 130 135 140

Ser Ala Trp Val Gly Ile Asp Gly Asp Thr Tyr Gly Asn Ala Ile Leu
 145 150 155 160

Gln Thr Gly Val Asp Phe Thr Val Thr Asp Gly Glu Ala Ser Phe Asp
 165 170 175

Ala Trp Tyr Glu Trp Tyr Pro Asp Tyr Ala Tyr Asp Phe Ser Gly Ile
180 185 190

Asp Ile Ser Ala Gly Asp Glu Ile Val Ala Ile Val Glu Ser Tyr Thr
195 200 205

Ser Thr Thr Gly Ile Ala Ile Ile Glu Asn Lys Ser Thr Gly Gln Lys
210 215 220

Val Ser Lys Glu Leu Ser Ser Ser Ser Ser Leu Gly Gly Gln Asn Ala
225 230 235 240

Glu Trp Ile Val Glu Asp Phe Glu Glu Asn Gly Ser Leu Val Asn Leu
245 250 255

Val Asp Phe Gly Thr Val Thr Phe Thr Gly Ala Val Ala Lys Ala Ala
260 265 270

Gly Gly Glu Ser Val Gly Leu Thr Asp Ala Thr Ile Ile Glu Ile Glu
275 280 285

Glu Asn Gly Gln Val Val Thr Asp Val Thr Ile Asp Ser Asp Ser Glu
290 295 300

Val Thr Ile Thr Tyr Glu
305 310

<210> 159
<211> 681
<212> PRT
<213> *Aspergillus niger*

<400> 159

Met Arg Cys Ser Leu Ile Ser Leu Leu Gly Leu Ala Ala Ile Pro Ala
1 5 10 15

Leu Gly Gly Cys Pro Phe Ala His Thr Ala Asn Met Gly Ile Asp Asn
20 25 30

Met Val Lys Ala His Ala His Met Ser Arg Pro Leu Ile Ala Ser Lys
35 40 45

Ser Ser Pro Ser Thr Val Pro Thr Ser Ser Ser Thr Pro Ser Val Gly
 50 55 60

Gln Lys Gly Val Phe Met Met Asn Arg Ile Ala Pro Gly Thr Ser Glu
 65 70 75 80

Leu Tyr Ile Ala Asn Thr Asp Gly Ser Asn Glu Arg Pro Leu Leu Ser
 85 90 95

Asn Pro Val Tyr Glu Tyr His Ala Ser Phe Ser Pro Asp Val Glu Trp
 100 105 110

Ile Thr Phe Thr Ser Glu Arg Asn Gly Asp Gly Asn Ser Asp Ile Tyr
 115 120 125

Arg Val Arg Thr Asn Gly Ser Asp Leu Gln Glu Leu Val Ala Thr Pro
 130 135 140

Ala Val Glu Asp Ser Val Val Ile Ser Pro Asn Gly Arg Leu Ala Ala
 145 150 155 160

Tyr Val Ser Thr Ala Asn Asn Met Lys Ala Asn Ile Trp Ile Leu Asp
 165 170 175

Leu Gln Thr Gly Ala Gln Trp Asn Leu Thr Asn Thr Pro Thr Thr Ala
 180 185 190

Ala Asn Ser Ser Leu Met Glu Ser Tyr Leu Arg Pro Ala Trp Ser Pro
 195 200 205

Asp Gly Glu Trp Ile Ala Phe Ser Ser Asp Arg Asn Thr Gln Trp Asp
 210 215 220

Gly His Gly Val Pro Thr Phe Leu Gly Arg Thr Gly Trp Glu Thr Thr
 225 230 235 240

Gln Glu Leu Ser Leu Tyr Ala Ile Arg Pro Asn Gly Ser Asp Phe Arg
 245 250 255

Gln Ile Ile Ser Lys Pro Tyr Tyr Ser Leu Gly Ser Pro Lys Trp Ser
 260 265 270

Ala Asp Gly Lys Arg Ile Val Tyr Tyr Glu Met Thr Arg Glu Asp Thr

275 280 285
 Tyr Asn Ala His Arg Pro Glu Thr Ile Thr Thr Ala Asn Ser Thr Ile
 290 295 300
 Met Ser Val Asp Phe Glu Thr Gly Thr Asp Val Arg Val Glu Val Ala
 305 310 315 320
 Gly Ser Gly Val Lys Gln Phe Pro Gln Tyr Leu Asp Lys Asn Gly Thr
 325 330 335
 Ile Ala Tyr Thr Leu Lys Gly Gly Thr Ser Glu Gly Phe Tyr Thr Thr
 340 345 350
 Ala Gly Leu Tyr Val Asn Thr Thr Ser Ala Thr Leu Arg Ser Pro Ala
 355 360 365
 Trp Ser Pro Asp Gly Lys Gln Val Val Tyr Glu Lys Ser Thr Trp Ser
 370 375 380
 Ile Arg Ser Gly Tyr Lys Gln Leu Tyr Ser Trp Asp Ser Asp Trp Asp
 385 390 395 400
 Tyr Arg Phe Thr Asp Val Phe Pro Gln Val Ser His Gln Glu Arg Val
 405 410 415
 Ala Ile Thr Gln Lys Gln Leu Gly Asn Ser Ser Ile Val Thr Leu Asn
 420 425 430
 Thr Thr Gly Gly Asp Leu Gln Leu Val Tyr Asp Pro Ser Thr Ala Asp
 435 440 445
 Phe Val Ser Asp Asp Glu Thr Thr Gly Leu Ser Ala Tyr Gln Pro Ser
 450 455 460
 Trp Ser Pro Cys Gly Glu Trp Leu Val Phe Gly Val Gly Phe Trp Phe
 465 470 475 480
 Glu Thr Arg Glu Ala Ser Gly Gly Trp Ile Val Arg Ala Thr Ala Asn
 485 490 495
 Gly Ser Tyr Ser Glu Val Leu Val Asn Ser Ser Tyr Ser Ile Thr Glu
 500 505 510

Asp Gly Ala Leu Asn Ser Gly Phe Pro Ser Phe Ser Pro Asp Gly Lys
 515 520 525

Lys Val Val Tyr Arg Val Trp Gly Ala Asp Thr Ala Thr Tyr Gly Asn
 530 535 540

Ala Ser Glu Ile Gly Leu Arg Val Leu Asp Leu Glu Thr Arg Lys Thr
 545 550 555 560

Thr Val Leu Thr Thr Glu Trp Asp Asn Leu Pro Gln Phe Ser Pro Asp
 565 570 575

Gly Glu Leu Ile Leu Phe Thr Arg Lys Thr Ser Thr Tyr Asn Tyr Asp
 580 585 590

Val Cys Thr Ile Arg Pro Asp Gly Thr Asp Leu Arg Val Leu Thr Ser
 595 600 605

Ser Gly Ala Asn Asp Ala His Ala Val Trp Ser Gln Asp Gly Arg Ile
 610 615 620

Met Trp Ser Thr Gly Met Tyr Gly Phe Arg Phe Glu Cys Ala Leu Tyr
 625 630 635 640

Gly Asp Thr Phe Gln Pro Tyr Gly Gln Val Met Ile Met Asp Ala Asp
 645 650 655

Gly Gly Asn Lys Lys Leu Met Thr Asn Ser Met Trp Glu Asp Ser Met
 660 665 670

Pro Leu Phe Leu Pro Arg Glu Val Leu
 675 680

<210> 160

<211> 624

<212> PRT

<213> Aspergillus niger

<400> 160

Met Pro Pro Asp Ala Lys Ser Pro Gly Tyr Gln Pro Gly Met Ala Val
 1 5 10 15

Leu Pro Ser Arg Pro His Pro Ala Lys Gly Lys Ala Ile Arg Phe Leu
 20 25 30

Leu Ser Leu Ala Leu Val Ala Phe Ala Ile Val Gln Leu Cys Gly Asn
 35 40 45

Phe His Lys Asn Arg Ser Val Glu Gln Gln Leu Gln Ser Gln Thr Leu
 50 55 60

Asp Asp Glu Ser Phe Lys Trp Glu Asp Val Thr Pro Thr Lys Gln Leu
 65 70 75 80

Val Tyr His Pro Cys Phe Gly Asp His Glu Cys Ala Arg Leu Ser Leu
 85 90 95

Pro Met Asn Trp Asn Arg Thr Asp Gly Glu Gly Ser Lys Ile Ala Leu
 100 105 110

Ala Val Ile Lys Leu Pro Ala Lys Val Pro Val Thr Asp Ala Arg Tyr
 115 120 125

Gly Gly Ala Ile Leu Leu Asn Pro Gly Gly Pro Gly Gly Ser Gly Val
 130 135 140

Ser Met Val Phe Arg Tyr Gly Lys Ala Ile Gln Thr Ile Val Asp Ser
 145 150 155 160

Pro Glu Ser Pro Ser Ala Asp Ser Ala Ser Gly Lys Tyr Phe Asp Val
 165 170 175

Val Ser Phe Asp Pro Arg Gly Val Asn Asn Thr Thr Pro Asn Phe Ser
 180 185 190

Cys Phe Pro Asp Pro Ala Thr Arg Lys Ala Trp Leu Leu Gln Ser Glu
 195 200 205

Ala Glu Gly Leu Leu Gly Ser Ser Glu Gly Val Phe Asp Thr Arg Trp
 210 215 220

Ala Arg Tyr Glu Ala Phe Glu Arg Leu Leu Ser Thr Ala Pro Asn Thr
 225 230 235 240

Phe Pro Val Gly Thr Asn Val Asp Ala Glu Arg Ile Arg Leu His Asn

	245		250		255
Arg Trp Lys Lys Gly Glu Glu Lys Leu Leu Tyr Trp Gly Phe Ser Tyr	260		265		270
Gly Thr Ile Leu Gly Ser Thr Phe Ala Ala Met Gln Pro His Arg Ile	275		280		285
Asn Arg Ala Val Ile Asp Gly Val Cys Asn Ala Asp Asp Tyr Tyr Ala	290		295		300
Gly Asn Trp Leu Thr Asn Leu Gln Asp Ser Asp Ala Ala Phe Asn Lys	305		310		315
Phe Phe Glu Tyr Cys Tyr Thr Ala Gly Pro Ser Ala Cys Pro Phe Ala	325		330		335
Leu Gly Gly Asp Pro Glu Asp Leu Lys Ser Arg Tyr Glu Gln Ile Leu	340		345		350
Thr Asn Leu Thr Ser Ser Pro Ile Ala Val Ser Pro Ser Gly Asn Arg	355		360		365
Gly Pro Glu Ile Ile Thr Tyr Ser Asp Val Lys Ser Leu Val Val Gln	370		375		380
Ala Leu Tyr Val Pro Leu Lys Leu Phe Asp Leu Val Ala Arg Leu Leu	385		390		395
Ala Glu Leu Glu Gln Gly Asn Gly Ser Ser Phe Ala Asp Leu Lys Tyr	405		410		415
Glu Ala Lys Gln Trp Pro Val Pro Pro Pro Cys Asp Ser Ser Ser Thr	420		425		430
Gln Tyr Lys Val Pro Gly Glu Ser Asp Gln Glu Ala Gly Arg Asn Ile	435		440		445
Leu Cys Thr Asp Gly Pro Gly Leu Asp Gly Thr Ala Lys Glu Asp Phe	450		455		460
Arg Ser Tyr Trp Asn Met Leu Arg Gly Gln Ser Lys Ala Val Gly Asp	465		470		475
					480

Phe Trp Ala Glu Val Arg Met Ser Cys Val Lys Leu Glu Thr Arg Pro
485 490 495

Glu Trp Arg Tyr Asp Gly Met Arg Ile Gln Gly Pro Phe Ala Gly Asn
500 505 510

Thr Ser His Pro Leu Leu Phe Ile Gly Asn Thr Tyr Asp Pro Val Thr
515 520 525

Pro Leu Arg Asn Ala His Thr Met Ala Arg Gly Phe Pro Glu Ser Ile
530 535 540

Val Leu Glu Gln Asn Ser Val Gly His Cys Thr Leu Ser Gly Pro Ser
545 550 555 560

Leu Cys Thr Ala Lys Ala Ile Arg Gln Tyr Phe Gln Thr Gly Glu Leu
565 570 575

Pro Asp Pro Gly Thr Val Cys Gln Val Glu Glu Leu Pro Phe Arg Leu
580 585 590

Ala Gly Tyr Glu Arg Ser Gln Val Met Ser Pro Gly Asp Thr Glu Leu
595 600 605

Met Ser Ala Leu His Ser Leu Ser Glu Phe Arg His Leu Leu Gly Ala
610 615 620

<210> 161

<211> 554

<212> PRT

<213> Aspergillus niger

<400> 161

Met Leu Ser Ser Leu Leu Leu Gly Gly Leu Leu Gly Leu Ala Thr Ala
1 5 10 15

Gln Phe Pro Pro Glu Pro Glu Gly Ile Thr Val Leu Lys Ser Lys Leu
20 25 30

His Glu Asn Val Thr Ile Ser Phe Lys Glu Pro Gly Ile Cys Glu Thr
35 40 45

Thr Pro Gly Val Arg Ser Tyr Ser Gly Tyr Val His Leu Pro Pro Ala
 50 55 60

Ser Thr Ser Phe Phe Trp Phe Phe Glu Ala Arg Lys Asp Pro Ser Asn
 65 70 75 80

Ala Pro Leu Ala Ile Trp Leu Asn Gly Gly Pro Gly Gly Ser Ser Leu
 85 90 95

Met Gly Leu Leu Glu Glu Leu Gly Pro Cys Ser Ile Ala Ser Asp Ser
 100 105 110

Lys Thr Thr Val Leu Asn Pro Trp Ser Trp Asn Asn Glu Val Asn Leu
 115 120 125

Leu Phe Leu Asp Gln Pro Thr Gln Val Gly Phe Ser Tyr Asp Val Pro
 130 135 140

Thr Asn Gly Thr Leu Thr Ala Asn Gly Thr Ala Phe Ala Ala His Ala
 145 150 155 160

Leu Trp His Phe Ala Gln Thr Trp Phe Phe Glu Phe Pro His Tyr Lys
 165 170 175

Pro Asn Asp Asp Arg Val Ser Leu Trp Ala Glu Ser Tyr Gly Gly His
 180 185 190

Tyr Gly Pro Gly Ile Phe Arg Phe Phe Gln Gln Gln Asn Asp Lys Ile
 195 200 205

Ala Glu Gly Thr Ala Glu Asp Gly Ala Gln Tyr Leu His Leu Asp Thr
 210 215 220

Leu Gly Ile Val Asn Gly Leu Met Asp Met Val Ile Gln Glu Glu Ala
 225 230 235 240

Tyr Ile Thr Trp Pro Tyr Asn Asn Val Arg Leu Ala Pro Ser Ser Phe
 245 250 255

Asn Ser Arg Gly Phe Arg Asp Gln Ala Leu Ala Cys Glu Ala Ala Leu
 260 265 270

Lys Glu Arg Asp Ser Gly Leu Pro His Ser Gly Lys Asn Ile Ser Glu

275	280	285
Ile Cys Gly Gly Leu Ala Leu Glu Trp Gly Asp Gly Pro Ile Thr Tyr 290 295 300		
Tyr His Thr Phe Asn Arg Gly Trp Tyr Asp Ile Ala His Pro Lys Asn 305 310 315 320		
Asp Pro Phe Pro Ala Lys His Met Leu Gly Tyr Leu Thr Gln Glu Ser 325 330 335		
Val Leu Ala Ala Leu Gly Val Pro Val Asn Phe Thr Ser Ser Ser Ser 340 345 350		
Ala Val Ala Thr Gln Phe Ile Lys Thr Phe Asp Ile Val His Gly Gly 355 360 365		
Phe Leu Asp Ala Ile Gly Tyr Leu Leu Asp Ser Gly Val Lys Val His 370 375 380		
Met Met Tyr Gly Asp Arg Asp Tyr Ala Cys Asn Trp Val Gly Gly Glu 385 390 395 400		
Lys Ala Ser Leu Ala Val Pro Tyr Ser Arg Ile Thr Glu Phe Ala Asp 405 410 415		
Thr Gly Tyr Ser Pro Leu Leu Thr Pro Asp Gly Ile Ser Gly Met Thr 420 425 430		
Arg Gln Leu Gly Asn Tyr Ser Phe Thr Arg Val Phe Gln Ala Gly His 435 440 445		
Glu Val Pro Ser Tyr Gln Pro Val Ala Ala Tyr Glu Ile Phe Met Arg 450 455 460		
Ala Thr Phe Asn Lys Asp Ile Pro Thr Gly Leu Leu Ala Val Asp Asp 465 470 475 480		
Glu Phe Gln Ser Val Gly Pro Lys Asp Thr Trp His Ile Lys Asn Ile 485 490 495		
Pro Pro Ile Met Pro Lys Pro Gln Cys Tyr Val Leu Ser Pro Gly Thr 500 505 510		

Cys Thr Pro Glu Val Trp Glu Thr Val Leu Asn Gly Ser Ala Thr Val
515 520 525

Lys Asp Trp Tyr Val Val Asp Asp Ser Ala Gly Val Glu Asp His Glu
530 535 540

Gly Phe Ser Ile Leu Gly Gly Asp Glu Leu
545 550

<210> 162
<211> 578
<212> PRT
<213> Aspergillus niger

<400> 162

Met Thr Arg Phe Gln Leu Leu Pro Leu Val Ala Gly Leu Leu Ala Pro
1 5 10 15

Ser Ile Ala Ala Leu Ser Ile Pro Ser Pro Gln Gln Ile Leu Asp Ser
20 25 30

Leu Thr Phe Gly Glu His Thr Asp Gly Phe Cys Pro Leu Ala Pro Lys
35 40 45

Val Glu Val Pro Asp Asp Gly Phe Phe Pro Ala Leu Lys Phe Val Glu
50 55 60

Asp Ala Ser Phe Lys Ser Arg Gln Val Asn Arg Leu Ser Arg Ala Val
65 70 75 80

Gln Val Pro Thr Ala Ile Asp Asp Tyr Met Lys Asp Pro Tyr Asp Glu
85 90 95

Lys Phe Ala Pro Phe Leu Asp Phe Gln Lys Leu Leu Gln Thr Leu Phe
100 105 110

Pro Leu Thr His Ser Tyr Ala Arg Val Asp His Ile Asn Arg Phe Gly
115 120 125

Leu Val Phe Thr Leu Asn Gly Thr Asp Asp Ser Leu Lys Pro Leu Leu
130 135 140

Phe Thr Ala His Gln Asp Val Val Pro Ile Asn Asp Pro Ala Asp Trp
 145 150 155 160

Thr Tyr Pro Pro Phe Asp Gly His Tyr Asp Gly Glu Trp Leu Trp Gly
 165 170 175

Arg Gly Ala Ser Asp Cys Lys Asn Val Leu Ile Gly Leu Met Ser Val
 180 185 190

Val Glu Asp Leu Leu Ser Gln Lys Trp Glu Pro Thr Arg Thr Val Val
 195 200 205

Leu Ala Phe Gly Phe Asp Glu Glu Ser His Gly Phe Leu Gly Ala Gly
 210 215 220

Ser Ile Ala Lys Phe Leu Glu Lys Lys Tyr Gly Pro Asp Ser Phe Glu
 225 230 235 240

Phe Ile Leu Asp Glu Gly Gly Met Gly Leu Glu Val Leu Asp Asp Asn
 245 250 255

Asn Asn Gly Val Val Tyr Ala Leu Pro Gly Val Gly Glu Lys Gly Ser
 260 265 270

Ile Asp Val Val Leu Thr Leu Ala Val Pro Gly Gly His Ser Ser Val
 275 280 285

Pro Pro Pro His Thr Gly Ile Gly Ile Ile Ala Glu Ile Ile Tyr Glu
 290 295 300

Leu Glu Arg Gln Asp Leu Phe Val Pro Val Leu Asp Thr His His Pro
 305 310 315 320

Thr Arg Lys Met Leu Glu Cys Gln Val Arg His Ser Pro Ser Gln Val
 325 330 335

Glu Pro Trp Leu Ala Ser Ala Leu Gln Ser Ser Asp Tyr Ile Ser Leu
 340 345 350

Ala Glu Lys Leu Ala Ser Ser Arg Gly Asp Lys Phe Arg Phe Ile Leu
 355 360 365

Gln Thr Ser Gln Ala Ala Asp Ile Ile Asn Gly Gly Val Lys Ser Asn

370 375 380
 Ala Leu Pro Glu Lys Ile Asn Ala Leu Val Asn Tyr Arg Ile Ala Leu
 385 390 395 400
 His Gln Thr Pro Asp Asp Ile Lys Asn Arg Ala Val Glu Ile Ile Ser
 405 410 415
 Pro Ile Val Lys Lys Tyr Asn Leu Ser Leu Thr Ala Phe Pro Glu Ser
 420 425 430
 Asp Thr Val Asp Pro Ser Leu Asn Asn His Leu Thr Leu Thr Thr Leu
 435 440 445
 Ser Gly Ala Leu Ser Pro Ala Pro Val Ser Pro Thr Asp Ile Asp Thr
 450 455 460
 Asp Ala Val Trp Ala Arg Phe Ser Gly Val Thr Arg Ser Val Phe Glu
 465 470 475 480
 Ser Val Pro Ser Leu Glu Gly Arg Lys Val Val Val Ser Gly Asp Ile
 485 490 495
 Met Thr Gly Asn Thr Asp Thr Arg Phe Tyr Trp Ala Leu Ser Arg Asn
 500 505 510
 Ile Tyr Arg Trp Ser Pro Ser Arg Ala Gly Lys Ala Leu Asn Ile His
 515 520 525
 Thr Val Asp Glu Arg Ile Asp Ile Asp Ile His Leu Glu Ala Met Met
 530 535 540
 Leu Tyr Tyr Asp Leu Ile Arg Ser Phe Asp Gly Arg Thr Asp Ser Ser
 545 550 555 560
 Val Ile Ser Ala Ala Ser Ala Ala Ala Asp Asp Glu Leu Ala His Asp
 565 570 575
 Val Leu

<210> 163
 <211> 456

<212> PRT

<213> Aspergillus niger

<400> 163

Met Lys Ser Thr Thr Leu Leu Ser Leu Ala Trp Ala Ala Gln Ser Ala
 1 5 10 15

Tyr Ser Leu Ser Ile His Glu Arg Asp Glu Pro Ala Thr Leu Gln Phe
 20 25 30

Asn Phe Glu Arg Arg Gln Ile Ala Asp Arg Ser Arg Arg Lys Arg Ser
 35 40 45

Thr Ala Ser Ala Asp Leu Val Asn Leu Ala Thr Asn Leu Gly Tyr Thr
 50 55 60

Met Asn Leu Thr Leu Gly Thr Pro Gly Gln Glu Val Ser Val Thr Leu
 65 70 75 80

Asp Thr Gly Ser Ser Asp Leu Trp Val Asn Gly Ala Asn Ser Ser Val
 85 90 95

Cys Pro Cys Thr Asp Tyr Gly Ser Tyr Asn Ser Ser Ala Ser Ser Thr
 100 105 110

Tyr Thr Phe Val Asn Asp Glu Phe Tyr Ile Gln Tyr Val Asp Gly Ser
 115 120 125

Glu Ala Thr Gly Asp Tyr Val Asn Asp Thr Leu Lys Phe Ser Asn Val
 130 135 140

Thr Leu Thr Asn Phe Gln Phe Ala Val Ala Tyr Asp Gly Asp Ser Glu
 145 150 155 160

Glu Gly Val Leu Gly Ile Gly Tyr Ala Ser Asn Glu Ala Ser Gln Ala
 165 170 175

Thr Val Gly Gly Gly Glu Tyr Thr Asn Phe Pro Glu Ala Leu Val Asp
 180 185 190

Gln Gly Ala Ile Asn Trp Pro Ala Tyr Ser Leu Trp Leu Asp Asp Leu
 195 200 205

Asp Glu Gly Lys Gly Thr Ile Leu Phe Gly Gly Val Asn Thr Ala Lys
 210 215 220
 Tyr Tyr Gly Ser Leu Gln Thr Leu Pro Ile Val Ser Ile Glu Asp Met
 225 230 235 240
 Tyr Val Glu Phe Ala Val Asn Leu Thr Ala Val His Leu Glu Lys Asn
 245 250 255
 Gly Asn Ser Val Ser Val Asn Asn Ser Ala Thr Gln Phe Pro Ile Pro
 260 265 270
 Ala Val Leu Asp Ser Gly Thr Ala Leu Thr Tyr Ile Pro Thr Ser Ala
 275 280 285
 Ala Ala Ser Ile Tyr Glu Ala Val Gly Ala Gln Tyr Leu Ser Glu Tyr
 290 295 300
 Gly Tyr Gly Val Ile Glu Cys Asp Val Lys Asp Glu Asp Phe Thr Phe
 305 310 315 320
 Leu Phe Asp Phe Gly Ser Phe Asn Met Ser Val Asp Ile Ser Glu Met
 325 330 335
 Ile Leu Glu Ala Ser Ser Asp Met Thr Asp Met Asn Val Cys Thr Phe
 340 345 350
 Gly Leu Ala Val Ile Glu Asn Glu Ala Leu Leu Gly Asp Thr Phe Leu
 355 360 365
 Arg Ser Ala Tyr Val Val Tyr Asp Leu Gly Asn Asn Glu Ile Ser Leu
 370 375 380
 Ala Lys Ala Asn Phe Asn Pro Gly Glu Asp His Val Leu Glu Ile Gly
 385 390 395 400
 Thr Gly Ser Asp Ala Val Pro Lys Ala Thr Gly Ala Thr Ala Thr Gly
 405 410 415
 Ala Ala Ala Thr Ser Thr Ala Ser Ser Asp Lys Ser Asp Lys Glu Ser
 420 425 430
 Ser Ala Thr Val Pro Arg Ser Gln Ile Val Ser Leu Val Ala Gly Val

435 440 445
 Leu Val Gly Val Phe Leu Val Leu
 450 455

 <210> 164
 <211> 664
 <212> PRT
 <213> *Aspergillus niger*

 <400> 164
 Met Leu Val Arg Gln Leu Ala Leu Ala Leu Ala Ile Ala Ala Leu Ser
 1 5 10 15

 Asp Ala Ile Pro Thr Ser Ile Lys His Val Leu His Glu Lys Arg His
 20 25 30

 Lys Pro Ala Ser Asp Trp Val Lys Gly Ala Arg Val Glu Ser Asp Ala
 35 40 45

 Val Leu Pro Met Arg Ile Gly Leu Ala Gln Asn Asn Leu Asp Lys Gly
 50 55 60

 Tyr Asp Phe Leu Met Glu Val Ser Asp Pro Lys Ser Ser Lys Tyr Gly
 65 70 75 80

 Gln Tyr Trp Ser Ala Asp Glu Val His Asp Ile Phe Ser Pro Ser Glu
 85 90 95

 Glu Ala Val Glu Ala Val Arg Glu Trp Leu Val Ala Ser Gly Ile His
 100 105 110

 Pro Ser Arg Val Val His Ser Asp Asn Lys Gly Trp Leu Ala Phe Asp
 115 120 125

 Ala Tyr Ala His Glu Ala Glu Arg Leu Phe Met Thr Glu Phe His Glu
 130 135 140

 His Glu Ser Asp Arg Ser Ala Lys Ile Arg Val Gly Cys Asp Gln Tyr
 145 150 155 160

 His Val Pro Glu His Ile Gln Lys His Ile Asp Tyr Ile Thr Pro Gly
 165 170 175

Val Lys Leu Thr Gln Val Val Lys Arg Thr Asn Lys Val Lys Arg Ala
 180 185 190

Ser Gln Leu Ala His Ser Ser Lys Ala Lys Ser Ala Ala Gln Gly Pro
 195 200 205

Gln Pro Leu Pro Asn Lys Ala Lys Phe Leu Pro Glu Asp Leu Arg Gly
 210 215 220

Cys Gly Tyr Asn Ile Thr Pro Ser Cys Ile Lys Ala Leu Tyr Gln Ile
 225 230 235 240

Pro Asp Ala Lys Thr Ala Thr Pro Asn Asn Ser Leu Gly Leu Tyr Glu
 245 250 255

Gln Gly Asp Tyr Phe Ala Lys Ser Asp Leu Asp Leu Phe Tyr Lys Glu
 260 265 270

Tyr Ala Pro Trp Val Pro Gln Gly Thr Tyr Pro Ile Pro Ala Leu Ile
 275 280 285

Asp Gly Ala Asn Tyr Ser Val Pro Ser Tyr Ser Ser Leu Asn Thr Gly
 290 295 300

Glu Ser Asp Ile Asp Ile Asp Met Ala Tyr Ser Leu Leu Tyr Pro Gln
 305 310 315 320

Gln Val Thr Leu Tyr Gln Val Asp Asp Gln Leu Tyr Glu Pro Val Glu
 325 330 335

Val Asp Thr Thr Asn Leu Phe Asn Thr Phe Leu Asp Ala Leu Asp Gly
 340 345 350

Ser Tyr Cys Thr Tyr Ser Ala Tyr Gly Glu Thr Gly Asp Asp Pro Ser
 355 360 365

Ile Asp Pro Val Tyr Pro Asp Thr Arg Pro Gly Gly Tyr Lys Gly Lys
 370 375 380

Leu Gln Cys Gly Val Tyr Lys Pro Thr Asn Val Ile Ser Ala Ser Tyr
 385 390 395 400

Gly Gln Ser Glu Ala Asp Leu Pro Val Ser Tyr Thr Lys Arg Gln Cys
405 410 415

Asn Glu Phe Met Lys Leu Gly Leu Gln Gly His Ser Ile Leu Phe Ala
420 425 430

Ser Gly Asp Tyr Gly Val Ala Ser Phe Ala Gly Asp Gly Asp Glu Asn
435 440 445

Gly Cys Leu Gly Pro Glu Gly Lys Ile Phe Asn Pro Gln Tyr Pro Ser
450 455 460

Asn Cys Pro Tyr Val Thr Ser Val Gly Gly Thr Met Leu Tyr Gly Tyr
465 470 475 480

Gln Thr Val Asn Asp Ser Glu Ser Val Met His Val Asn Leu Gly Gly
485 490 495

Thr Ala Ser Asn Phe Ser Thr Ser Gly Gly Phe Ser Asn Tyr Phe Pro
500 505 510

Gln Pro Ala Tyr Gln Phe Ala Ala Val Glu Gln Tyr Phe Gln Ser Ala
515 520 525

Asn Leu Ser Tyr Pro Tyr Tyr Ser Glu Phe Glu Val Asp Val Asn Thr
530 535 540

Thr Lys Gly Leu Tyr Asn Arg Leu Gly Arg Ala Tyr Pro Asp Val Ser
545 550 555 560

Ala Asn Gly Ala His Phe Arg Ala Tyr Met Asp Gly Tyr Asp Tyr His
565 570 575

Trp Tyr Gly Ser Ser Leu Ala Ser Pro Leu Phe Ala Ser Val Leu Thr
580 585 590

Leu Leu Asn Glu Glu Arg Phe Ala Ile Gly Lys Gly Pro Val Gly Phe
595 600 605

Val	Asn	Pro	Val	Leu	Tyr	Ala	Tyr	Pro	Gln	Val	Leu	Asn	Asp	Ile	Thr
610						615					620				

Asn Gly Thr Asn Ala Gly Cys Gly Thr Tyr Gly Phe Ser Ala Ile Glu

625 630 635 640
 Gly Trp Asp Pro Ala Ser Gly Leu Gly Thr Pro Asn Tyr Pro Leu Met
 645 650 655
 Lys Glu Leu Phe Leu Ser Leu Pro
 660

 <210> 165
 <211> 520
 <212> PRT
 <213> Aspergillus niger

 <400> 165
 Met Arg Val Thr Thr Ala Ile Ala Ser Leu Leu Leu Val Gly Ser Ala
 1 5 10 15

 Thr Ser Leu Gln Asn Pro His Arg Arg Ala Val Pro Pro Pro Leu Ser
 20 25 30

 His Arg Ser Val Ala Ser Arg Ser Val Pro Val Glu Arg Arg Thr Thr
 35 40 45

 Asp Phe Glu Tyr Leu Thr Asn Lys Thr Ala Arg Phe Leu Val Asn Gly
 50 55 60

 Thr Ser Ile Pro Glu Val Asp Phe Asp Val Gly Glu Ser Tyr Ala Gly
 65 70 75 80

 Leu Leu Pro Asn Thr Pro Thr Gly Asn Ser Ser Leu Phe Phe Trp Phe
 85 90 95

 Phe Pro Ser Gln Asn Pro Glu Ala Ser Asp Glu Ile Thr Ile Trp Leu
 100 105 110

 Asn Gly Gly Pro Gly Cys Ser Ser Leu Asp Gly Leu Leu Gln Glu Asn
 115 120 125

 Gly Pro Phe Leu Trp Gln Pro Gly Thr Tyr Lys Pro Val Pro Asn Pro
 130 135 140

 Tyr Ser Trp Thr Asn Leu Thr Asn Val Val Tyr Ile Asp Gln Pro Ala
 145 150 155 160

Gly Thr Gly Phe Ser Pro Gly Pro Ser Thr Val Asn Asn Glu Glu Asp
 165 170 175
 Val Ala Ala Gln Phe Asn Ser Trp Phe Lys His Phe Val Asp Thr Phe
 180 185 190
 Asp Leu His Gly Arg Lys Val Tyr Ile Thr Gly Glu Ser Tyr Ala Gly
 195 200 205
 Met Tyr Val Pro Tyr Ile Ala Asp Ala Met Leu Asn Glu Glu Asp Thr
 210 215 220
 Thr Tyr Phe Asn Leu Lys Gly Ile Gln Ile Asn Asp Pro Ser Ile Asn
 225 230 235 240
 Ser Asp Ser Val Met Met Tyr Ser Pro Ala Val Arg His Leu Asn His
 245 250 255
 Tyr Asn Asn Ile Phe Gln Leu Asn Ser Thr Phe Leu Ser Tyr Ile Asn
 260 265 270
 Ala Lys Ala Asp Lys Cys Gly Tyr Asn Ala Phe Leu Asp Lys Ala Ile
 275 280 285
 Thr Tyr Pro Pro Pro Ser Pro Phe Pro Thr Ala Pro Glu Ile Thr Glu
 290 295 300
 Asp Cys Gln Val Trp Asp Glu Val Val Met Ala Ala Tyr Asp Ile Asn
 305 310 315 320
 Pro Cys Phe Asn Tyr Tyr His Leu Ile Asp Phe Cys Pro Tyr Leu Trp
 325 330 335
 Asp Val Leu Gly Phe Pro Ser Leu Ala Ser Gly Pro Asn Asn Tyr Phe
 340 345 350
 Asn Arg Ser Asp Val Gln Lys Ile Leu His Val Pro Pro Thr Asp Tyr
 355 360 365
 Ser Val Cys Ser Glu Thr Val Ile Phe Ala Asn Gly Asp Gly Ser Asp
 370 375 380

Pro Ser Ser Trp Gly Pro Leu Pro Ser Val Ile Glu Arg Thr Asn Asn
385 390 395 400

Thr Ile Ile Gly His Gly Trp Leu Asp Tyr Leu Leu Phe Leu Asn Gly
405 410 415

Ser Leu Ala Thr Ile Gln Asn Met Thr Trp Asn Gly Lys Gln Gly Phe
420 425 430

Gln Arg Pro Pro Val Glu Pro Leu Phe Val Pro Tyr His Tyr Gly Leu
435 440 445

Ala Glu Leu Tyr Trp Gly Asp Glu Pro Asp Pro Tyr Asn Leu Asp Ala
450 455 460

Gly Ala Gly Tyr Leu Gly Thr Ala His Thr Glu Arg Gly Leu Thr Phe
465 470 475 480

Ser Ser Val Tyr Leu Ser Gly His Glu Ile Pro Gln Tyr Val Pro Gly
485 490 495

Ala Ala Tyr Arg Gln Leu Glu Phe Leu Leu Gly Arg Ile Ser Ser Leu
500 505 510

Ser Ala Lys Gly Asn Tyr Thr Ser
515 520

<210> 166
<211> 551
<212> PRT
<213> Aspergillus niger

<400> 166

Met Arg Gly Ser Arg Leu Val Leu Leu Leu Pro Leu Ala Ala Leu Ser
1 5 10 15

Cys Ala Met Pro Glu Asn Glu Trp Ser Ser Thr Ile Arg Arg Gln Leu
20 25 30

Pro Lys Ala Ser Thr Gly Val Lys Ser Ile Lys Thr Pro Asn Asn Val
35 40 45

Thr Ile Arg Tyr Lys Glu Pro Gly Thr Glu Gly Ile Cys Glu Thr Thr
50 55 60

Pro Gly Val Lys Ser Tyr Ser Gly Tyr Val Asp Leu Ser Pro Glu Ser
65 70 75 80

His Thr Phe Phe Trp Phe Phe Glu Ser Arg Arg Asp Pro Glu Asn Asp
85 90 95

Pro Val Thr Leu Trp Leu Asn Gly Gly Pro Gly Ser Asp Ser Leu Ile
100 105 110

Gly Leu Phe Glu Glu Leu Gly Pro Cys His Ile Thr Pro Glu Tyr Glu
115 120 125

Ser Ile Ile Asn Gln Tyr Ser Trp Asn Glu Val Thr Asn Leu Leu Phe
130 135 140

Leu Ser Gln Pro Leu Gly Val Gly Phe Ser Tyr Ser Glu Thr Glu Ala
145 150 155 160

Gly Ser Leu Asn Pro Phe Thr Gly Ala Val Glu Asn Ala Ser Phe Ala
165 170 175

Gly Val Gln Gly Arg Tyr Pro Val Ile Asp Ala Thr Ile Ile Asp Thr
180 185 190

Thr Asp Ile Ala Ala Arg Ala Thr Trp Glu Val Leu Gln Gly Phe Leu
195 200 205

Ser Gly Leu Ser Gln Leu Asp Ser Glu Val Lys Ser Lys Glu Phe Asn
210 215 220

Leu Trp Thr Glu Ser Tyr Gly Gly His Tyr Gly Pro Ala Phe Phe Asn
225 230 235 240

His Phe Tyr Glu Gln Asn Ser Lys Ile Ala Ser Gly Glu Val Asn Gly
245 250 255

Val Gln Leu Asn Phe Asn Ser Leu Gly Ile Ile Asn Gly Ile Ile Asp
260 265 270

Ala Ala Ile Gln Ala Asp Tyr Tyr Ala Asp Phe Ala Val Asn Asn Thr
275 280 285

Tyr Gly Ile Lys Ala Val Asn Asp Thr Val Tyr Asn Tyr Met Lys Phe
 290 295 300
 Ala Asn Thr Met Pro Asn Gly Cys Gln Asp Gln Val Ala Ser Cys Lys
 305 310 315 320
 Leu Thr Asn Arg Thr Ser Leu Ser Asp Tyr Ala Ile Cys Thr Glu Ala
 325 330 335
 Ala Asn Met Cys Arg Asp Asn Val Glu Gly Pro Tyr Tyr Gln Phe Gly
 340 345 350
 Gly Arg Gly Val Tyr Asp Ile Arg His Pro Tyr Asn Asp Pro Thr Pro
 355 360 365
 Pro Ser Tyr Phe Val Asp Tyr Leu Lys Lys Asp Ser Val Met Asp Ala
 370 375 380
 Ile Gly Val Asp Ile Asn Tyr Thr Glu Ser Ser Gly Glu Val Tyr Tyr
 385 390 395 400
 Ala Phe Gln Gln Thr Gly Asp Phe Val Trp Pro Asn Phe Ile Glu Asp
 405 410 415
 Leu Glu Glu Ile Leu Gln Leu Pro Val Arg Val Ser Leu Ile Tyr Gly
 420 425 430
 Asp Ala Asp Tyr Ile Cys Asn Trp Phe Gly Gly Gln Ala Ile Ser Leu
 435 440 445
 Ala Val Asn Tyr Pro His Ala Ala Gln Phe Arg Ala Ala Gly Tyr Thr
 450 455 460
 Pro Met Thr Val Asp Gly Val Glu Tyr Gly Glu Thr Arg Glu Tyr Gly
 465 470 475 480
 Asn Phe Ser Phe Thr Arg Val Tyr Gln Ala Gly His Glu Val Pro Tyr
 485 490 495
 Tyr Gln Pro Ile Ala Ala Leu Gln Leu Phe Asn Arg Thr Leu Phe Gly
 500 505 510

Trp Asp Ile Ala Ala Gly Thr Thr Gln Ile Trp Pro Glu Tyr Ser Thr
515 520 525

Asn Gly Thr Ser Gln Ala Thr His Thr Glu Ser Phe Val Pro Leu Ser
530 535 540

Thr Ala Ser Ser Thr Val Asn
545 550

<210> 167
<211> 623
<212> PRT
<213> Aspergillus niger

<400> 167

Met Pro Phe Pro Phe Ser Ser Ala Leu Leu Gly Tyr Ile Leu Thr Thr
1 5 10 15

Ser Thr Thr Leu Thr Ser Leu Val Ala Gly Gln Tyr Tyr Pro Pro Thr
20 25 30

Pro Glu Asp Leu Thr Val Ile His Ser Glu Ile Phe Pro Gly Ala Arg
35 40 45

Ile Ser Tyr Lys Gln Pro Leu Gly Ile Cys Thr Thr Thr Pro Ser Thr
50 55 60

Pro Ser Tyr Ser Gly Tyr Ile His Leu Pro Pro His Thr Leu Thr Asn
65 70 75 80

Leu Ser Ile Pro Gly Ile Ser Ile Ser Gln Pro Tyr Pro Ile Asn Thr
85 90 95

Phe Phe Trp Tyr Phe Pro Ser Arg His His His Asn Asn Asp Thr Ser
100 105 110

Pro Leu Thr Ile Trp Met Asn Gly Gly Pro Gly Gly Ser Ser Met Ile
115 120 125

Gly Leu Phe Gln Glu Asn Gly Pro Cys Thr Val Asn Thr Asp Ser Asn
130 135 140

Ser Thr Ala Tyr Asn Pro Trp Ser Trp Asn Glu Tyr Val Asp Met Leu
145 150 155 160

Tyr Ile Glu Gln Pro Val Gln Thr Gly Phe Ser Tyr Asp Val Leu Arg
 165 170 175

Asn Gly Thr Leu Asp Leu Asn Glu Thr Phe Leu Val Gly Thr Leu Pro
 180 185 190

Ser Gln Asp Val His Gly Thr Val Asn Gly Thr Val Asn Gly Gly Arg
 195 200 205

Ala Leu Trp Val Ala Leu Gln Val Trp Leu Gly Glu Phe Ser Glu Tyr
 210 215 220

Val Ser Ser Val Asp Gly Asn Gly Gly Gly Asp Asp Arg Val Ser Ile
 225 230 235 240

Trp Thr Glu Ser Tyr Gly Gly Arg Tyr Gly Pro Ala Tyr Thr Ala Leu
 245 250 255

Phe Gln Glu Met Asn Glu Arg Ile Glu Ser Gly Glu Val Ser Thr Gly
 260 265 270

Lys Lys Ile His Leu Asp Thr Leu Gly Ile Ile Asn Gly Cys Val Asp
 275 280 285

Leu Leu Val Gln Val Pro Ser Phe Pro Glu Gln Ala Tyr Asn Asn Thr
 290 295 300

Tyr Gly Ile Glu Gly Ile Asn Arg Thr Leu Tyr Asp Arg Ala Met Asp
 305 310 315 320

Ser Trp Ser Lys Pro Gly Gly Cys Arg Asp Met Ile Ile Glu Cys Arg
 325 330 335

Asp Ala Gly Glu Leu Gly Asp Pro Leu Ile Ile Cys Glu Glu Ala Ser
 340 345 350

Asp Tyr Cys Ser Arg Glu Ile Lys Ser Leu Tyr Thr Asn Thr Ser Gly
 355 360 365

Arg Gly Tyr Tyr Asp Ile Ala His Phe Thr Pro Asp Ala Ala Leu Val
 370 375 380

Pro Tyr Phe Val Gly Phe Leu Asn Arg Pro Trp Val Gln Lys Ala Leu
385 390 395 400

Gly Val Pro Val Asn Tyr Thr Met Ser Ser Glu Ala Val Gly Asn Ser
405 410 415

Phe Ala Ser Thr Gly Asp Tyr Pro Arg Asn Asp Pro Arg Gly Met Ile
420 425 430

Gly Asp Ile Gly Tyr Leu Leu Asp Ser Gly Val Lys Val Ala Met Val
435 440 445

Tyr Gly Asp Arg Asp Tyr Ala Cys Pro Trp Arg Gly Gly Glu Asp Val
450 455 460

Ser Leu Leu Val Glu Tyr Glu Asp Ala Glu Lys Phe Arg Ala Ala Gly
465 470 475 480

Tyr Ala Glu Val Gln Thr Lys Ser Ser Tyr Val Gly Gly Leu Val Arg
485 490 495

Gln Tyr Gly Asn Phe Ser Phe Thr Arg Val Phe Gln Ala Gly His Glu
500 505 510

Val Pro Phe Tyr Gln Pro Glu Thr Ala Tyr Glu Ile Phe Asn Arg Ala
515 520 525

Gln Phe Asn Trp Asp Ile Ala Thr Gly Gly Ile Ser Leu Glu Gln Asn
530 535 540

Gln Ser Tyr Gly Thr Glu Gly Pro Ser Ser Thr Trp His Ile Lys Asn
545 550 555 560

Glu Val Pro Glu Ser Pro Glu Pro Thr Cys Tyr Leu Leu Ala Met Asp
565 570 575

Ser Thr Cys Thr Asp Glu Gln Arg Glu Arg Val Leu Ser Gly Asp Ala
580 585 590

Val Val Arg Asp Trp Val Val Val Asp Asp Ile Glu Ala Glu Ser Ser
595 600 605

Phe Ser Gly Val Gly Asp Gln Leu Ala Gln Val Pro Leu Gly His
 610 615 620

<210> 168
 <211> 439
 <212> PRT
 <213> Aspergillus niger

<400> 168

Met Arg Thr Ser Thr Leu Leu Leu Leu Trp Ser Thr Ala Gly Ala Ala
 1 5 10 15

Leu Ala Ser Pro Tyr Pro Leu Pro Asp Ser Gln Val Val Phe Ala Ala
 20 25 30

Asp His Glu Val Pro Asn Thr Gln Gly Lys His Val Val Asp Glu Ala
 35 40 45

Ile Leu Ser Ala Leu Asn Ala His Ser Asp Pro Val Ala Ala Met Val
 50 55 60

Ser Leu Arg Pro Glu Thr Ala Ala Phe Leu Ala Glu Pro Arg Leu Leu
 65 70 75 80

His Ile Arg Gly Glu Glu Lys Ala Glu Trp Met Thr Glu Gly Asp Lys
 85 90 95

Leu Arg Leu Arg Gln Arg Gly Lys Lys Phe Met Asp Ile Thr Glu His
 100 105 110

Gln Asp Phe Tyr Ala Glu Gln Ala Met Ala Ser Phe Ala Gly Asp Pro
 115 120 125

Asn Leu Pro Lys Leu Ser His Lys Gly Leu Val Lys Pro Leu Phe Ser
 130 135 140

Gln Ile Glu Thr Glu Arg Met His Asp Ile Leu Gln His Met Thr Ser
 145 150 155 160

Tyr Tyr Asn Arg Tyr Tyr Gly Asp Tyr His Gly Glu Met Ser Ser Glu
 165 170 175

Trp Leu His Asp Tyr Ile Ala Ala Ile Ile Ser Lys Ser Pro Phe Arg
 180 185 190

Thr His Ile Ser Leu Glu Tyr Phe Thr His Pro Phe Arg Gln Ser Ser
 195 200 205

Ile Ile Ala Arg Phe Glu Pro Lys Val Arg Ser Phe Ser Gln Pro Leu
 210 215 220

Thr Ile Ile Gly Ala His Gln Asp Ser Ala Asn Tyr Leu Phe Pro Leu
 225 230 235 240

Leu Pro Ala Pro Gly Ala Asp Asp Asp Cys Ser Gly Thr Val Ser Ile
 245 250 255

Leu Glu Ala Phe Arg Val Leu Ala Glu Asn Gly Tyr Thr Pro Lys Asp
 260 265 270

Gly Pro Val Glu Phe His Trp Tyr Ala Ala Glu Glu Ala Gly Leu Leu
 275 280 285

Gly Ser Gln Ala Ile Ala Arg Tyr Lys Lys Glu Gln Gly Ala Lys Ile
 290 295 300

Asp Ala Met Met Glu Phe Asp Met Thr Ala Phe Ile Ala Arg Asn Ala
 305 310 315 320

Thr Glu Thr Ile Gly Phe Val Ala Thr Gln Ala Asp Ala Ala Leu Thr
 325 330 335

Asn Trp Ala Leu Asn Leu Ser Arg Glu Tyr Ile Ser Ile Pro Ala Glu
 340 345 350

Val Tyr Glu Leu Gly Pro Asn Ala Gly Ser Asp Tyr Met Ser Tyr Thr
 355 360 365

Lys Leu Asn Tyr Pro Ala Ala Phe Ala Ser Glu Gly Asn Pro Leu Ala
 370 375 380

Gly Gly Ser Phe Pro Gly Glu Met Asp Pro Tyr Val His Gly Ile Lys
 385 390 395 400

Asp Arg Met Asp Val Asp Asp Glu Thr Gly Val Phe Ser Ile Glu His
 405 410 415

Met Ala Arg Phe Ser Glu Leu Ala Ile Ala Phe Val Val Glu Gln Ala
 420 425 430

Gly Trp Asp Asn Thr Trp Arg
 435

<210> 169
 <211> 526
 <212> PRT
 <213> Aspergillus niger

<400> 169

Met Arg Ser Phe Ser Val Val Ala Ala Ala Ser Leu Ala Leu Ser Trp
 1 5 10 15

Ala Ser Leu Ala Gln Ala Ala Arg Pro Arg Leu Val Pro Lys Pro Ile
 20 25 30

Ser Arg Pro Ala Ser Ser Lys Ser Ala Ala Thr Thr Gly Glu Ala Tyr
 35 40 45

Phe Glu Gln Leu Leu Asp His His Asn Pro Glu Lys Gly Thr Phe Ser
 50 55 60

Gln Arg Tyr Trp Trp Ser Thr Glu Tyr Trp Gly Gly Pro Gly Ser Pro
 65 70 75 80

Val Val Leu Phe Asn Pro Gly Glu Val Ser Ala Asp Gly Tyr Glu Gly
 85 90 95

Tyr Leu Thr Asn Asp Thr Leu Thr Gly Val Tyr Ala Gln Glu Ile Gln
 100 105 110

Gly Ala Val Ile Leu Ile Glu His Arg Tyr Trp Gly Asp Ser Ser Pro
 115 120 125

Tyr Glu Val Leu Asn Ala Glu Thr Leu Gln Tyr Leu Thr Leu Asp Gln
 130 135 140

Ser Ile Leu Asp Met Thr Tyr Phe Ala Glu Thr Val Lys Leu Gln Phe
 145 150 155 160

Asp Asn Ser Ser Arg Ser Asn Ala Gln Asn Ala Pro Trp Val Met Val

	165		170		175
Gly Gly Ser Tyr Ser Gly Ala Leu Thr Ala Trp Thr Glu Ser Ile Ala	180		185		190
Pro Gly Thr Phe Trp Ala Tyr His Ala Thr Ser Ala Pro Val Glu Ala	195		200		205
Ile Tyr Asp Phe Trp Gln Tyr Phe Tyr Pro Ile Gln Gln Gly Met Ala	210		215		220
Gln Asn Cys Ser Lys Asp Val Ser Leu Val Ala Glu Tyr Val Asp Lys	225		230		235
Ile Gly Lys Asn Gly Thr Ala Lys Glu Gln Gln Glu Leu Lys Glu Leu	245		250		255
Phe Gly Leu Gly Ala Val Glu His Tyr Asp Asp Phe Ala Ala Val Leu	260		265		270
Pro Asn Gly Pro Tyr Leu Trp Gln Asp Asn Asp Phe Val Thr Gly Tyr	275		280		285
Ser Ser Phe Phe Gln Phe Cys Asp Ala Val Glu Gly Val Glu Ala Gly	290		295		300
Ala Ala Val Thr Pro Gly Pro Glu Gly Val Gly Leu Glu Lys Ala Leu	305		310		315
Ala Asn Tyr Ala Asn Trp Phe Asn Ser Thr Ile Leu Pro Asn Tyr Cys	325		330		335
Ala Ser Tyr Gly Tyr Trp Thr Asp Glu Trp Ser Val Ala Cys Phe Asp	340		345		350
Ser Tyr Asn Ala Ser Ser Pro Ile Phe Thr Asp Thr Ser Val Gly Asn	355		360		365
Pro Val Asp Arg Gln Trp Glu Trp Phe Leu Cys Asn Glu Pro Phe Phe	370		375		380
Trp Trp Gln Asp Gly Ala Pro Glu Gly Thr Ser Thr Ile Val Pro Arg	385		390		395
					400

Leu Val Ser Ala Ser Tyr Trp Gln Arg Gln Cys Pro Leu Tyr Phe Pro
 405 410 415

Glu Val Asn Gly Tyr Thr Tyr Gly Ser Ala Lys Gly Lys Asn Ser Ala
 420 425 430

Thr Val Asn Ser Trp Thr Gly Gly Trp Asp Met Thr Arg Asn Thr Thr
 435 440 445

Arg Leu Ile Trp Thr Asn Gly Gln Tyr Asp Pro Trp Arg Asp Ser Gly
 450 455 460

Val Ser Ser Thr Phe Arg Pro Gly Gly Pro Leu Val Ser Thr Ala Asn
 465 470 475 480

Glu Pro Val Gln Ile Ile Pro Gly Gly Phe His Cys Ser Asp Leu Tyr
 485 490 495

Met Glu Asp Tyr Tyr Ala Asn Glu Gly Val Arg Lys Val Val Asp Asn
 500 505 510

Glu Val Lys Gln Ile Lys Glu Trp Val Glu Glu Tyr Tyr Ala
 515 520 525

<210> 170
 <211> 424
 <212> PRT
 <213> Aspergillus niger

<400> 170

Met Gln Leu Leu Gln Ser Leu Ile Val Ala Val Cys Phe Ser Tyr Gly
 1 5 10 15

Val Leu Ser Leu Pro His Gly Pro Ser Asn Gln His Lys Ala Arg Ser
 20 25 30

Phe Lys Val Glu Arg Val Arg Arg Gly Thr Gly Ala Leu His Gly Pro
 35 40 45

Ala Ala Leu Arg Lys Ala Tyr Arg Lys Tyr Gly Ile Ala Pro Ser Ser
 50 55 60

Phe Asn Ile Asp Leu Ala Asp Phe Lys Pro Ile Thr Thr Thr His Ala
 65 70 75 80
 Ala Ala Gly Ser Glu Ile Ala Glu Pro Asp Gln Thr Gly Ala Val Ser
 85 90 95
 Ala Thr Ser Val Glu Asn Asp Ala Glu Phe Val Ser Pro Val Leu Ile
 100 105 110
 Gly Gly Gln Lys Ile Val Met Thr Phe Asp Thr Gly Ser Ser Asp Phe
 115 120 125
 Trp Val Phe Asp Thr Asn Leu Asn Glu Thr Leu Thr Gly His Thr Glu
 130 135 140
 Tyr Asn Pro Ser Asn Ser Ser Thr Phe Lys Lys Met Asp Gly Tyr Thr
 145 150 155 160
 Phe Asp Val Ser Tyr Gly Asp Asp Ser Tyr Ala Ser Gly Pro Val Gly
 165 170 175
 Thr Asp Thr Val Asn Ile Gly Gly Ala Ile Val Lys Glu Gln Ala Phe
 180 185 190
 Gly Val Pro Asp Gln Val Ser Gln Ser Phe Ile Glu Asp Thr Asn Ser
 195 200 205
 Asn Gly Leu Val Gly Leu Gly Phe Ser Ser Ile Asn Thr Ile Lys Pro
 210 215 220
 Glu Ala Gln Asp Thr Phe Phe Ala Asn Val Ala Pro Ser Leu Asp Glu
 225 230 235 240
 Pro Val Met Thr Ala Ser Leu Lys Ala Asp Gly Val Gly Glu Tyr Glu
 245 250 255
 Phe Gly Thr Ile Asp Lys Asp Lys Tyr Gln Gly Asn Ile Ala Asn Ile
 260 265 270
 Ser Val Asp Ser Ser Asn Gly Tyr Trp Gln Phe Ser Thr Pro Lys Tyr
 275 280 285
 Ser Val Ala Asp Gly Glu Leu Lys Asp Ile Gly Ser Leu Asn Thr Ser

290 295 300
 Ile Ala Asp Thr Gly Thr Ser Leu Met Leu Leu Asp Glu Asp Val Val
 305 310 315 320
 Thr Ala Tyr Tyr Ala Gln Val Pro Asn Ser Val Tyr Val Ser Ser Ala
 325 330 335
 Gly Gly Tyr Ile Tyr Pro Cys Asn Thr Thr Leu Pro Ser Phe Ser Leu
 340 345 350
 Val Leu Gly Glu Ser Ser Leu Ala Thr Ile Pro Gly Asn Leu Ile Asn
 355 360 365
 Phe Ser Lys Val Gly Thr Asn Thr Thr Thr Gly Gln Ala Leu Cys Phe
 370 375 380
 Gly Gly Ile Gln Ser Asn Gly Asn Thr Ser Leu Gln Ile Leu Gly Asp
 385 390 395 400
 Ile Phe Leu Lys Ala Phe Phe Val Val Phe Asp Met Arg Gly Pro Ser
 405 410 415
 Leu Gly Val Ala Ser Pro Lys Asn
 420
 <210> 171
 <211> 548
 <212> PRT
 <213> Aspergillus niger
 <400> 171
 Met Arg Ile Asp Ser Ala Ala Leu His Leu Val Pro Val Leu Leu Gly
 1 5 10 15
 Gln Val Gly Ala Leu Gln Leu Pro Leu Val Gln Asp Ser Asn Ser Gln
 20 25 30
 Trp Gln Lys Pro Asn Ala Gly Asp Lys Pro Leu Ile Ser Ser Pro Leu
 35 40 45
 Leu Gln Glu Gln Val Lys Ala Glu Asn Leu Leu Asp Arg Ala Arg Gln
 50 55 60

Leu Tyr Lys Ile Ala Glu Leu Gly Glu Asp Glu Tyr Asn His Pro Thr
 65 70 75 80

Arg Val Ile Gly Ser Lys Gly His Leu Gly Thr Leu Asp Tyr Ile Tyr
 85 90 95

Ser Thr Leu Thr Asp Leu Gly Asp Tyr Tyr Thr Val Val Asn Gln Ser
 100 105 110

Phe Pro Ala Val Ser Gly Asn Val Phe Glu Ser Arg Leu Val Leu Gly
 115 120 125

His Asp Val Pro Lys Ser Ala Thr Pro Met Gly Leu Thr Pro Pro Thr
 130 135 140

Arg Asn Lys Glu Pro Val Tyr Gly Ser Leu Val Ala Val Ser Asn Leu
 145 150 155 160

Gly Cys Glu Ala Ser Asp Tyr Ser Ser Asn Leu Lys Gly Ala Val Ala
 165 170 175

Phe Ile Ser Arg Gly Ser Cys Pro Phe Gly Thr Lys Ser Gln Leu Ala
 180 185 190

Gly Lys Ala Gly Ala Val Ala Ala Val Ile Tyr Asn Asn Glu Arg Gly
 195 200 205

Asp Leu Ser Gly Thr Leu Gly Asn Pro Thr Pro Asp His Val Ala Thr
 210 215 220

Phe Gly Ile Ser Asp Glu Asp Ala Ala Pro Val Leu Glu Lys Leu Asn
 225 230 235 240

Lys Gly Glu Lys Val Asp Ala Ile Ala Tyr Val Asp Ala Ile Val Glu
 245 250 255

Thr Ile His Thr Thr Asn Ile Ile Ala Gln Thr Thr Asp Gly Asp Pro
 260 265 270

Asn Asn Cys Val Met Leu Gly Gly His Ser Asp Ser Val Ala Glu Gly
 275 280 285

Pro Gly Ile Asn Asp Asp Gly Ser Gly Thr Leu Thr Leu Leu Glu Leu
 290 295 300

Ala Thr Leu Leu Thr Gln Phe Arg Val Asn Asn Cys Val Arg Phe Ala
 305 310 315 320

Trp Trp Ala Ala Glu Glu Gly Leu Leu Gly Ser Asp Tyr Tyr Val
 325 330 335

Ser Val Leu Thr Pro Glu Glu Asn Arg Lys Ile Arg Leu Phe Met Asp
 340 345 350

Tyr Asp Met Leu Gly Ser Pro Asn Phe Ala Tyr Gln Val Tyr Asn Ala
 355 360 365

Thr Asn Ala Val Asn Pro Glu Gly Ser Glu Glu Leu Arg Asp Leu Tyr
 370 375 380

Thr Asp Phe Tyr Glu Asp His Gly Phe Asn Tyr Thr Tyr Ile Pro Phe
 385 390 395 400

Asp Gly Arg Ser Asp Tyr Asp Ala Phe Ile Arg His Gly Ile Pro Gly
 405 410 415

Gly Gly Ile Ala Thr Gly Ala Glu Gly Ile Lys Thr Val Glu Glu Ala
 420 425 430

Asp Met Phe Gly Gly Val Ala Gly Gln Trp Tyr Asp Pro Cys Tyr His
 435 440 445

Gln Ile Cys Asp Thr Val Ala Asn Val Asn Leu Thr Ala Trp Glu Trp
 450 455 460

Asn Thr Lys Leu Val Ala His Ser Ile Ala Thr Tyr Ala Lys Ser Phe
 465 470 475 480

Asp Gly Phe Pro Glu Arg Ser Asp Glu Pro Ile Ser Pro Ala Ala Phe
 485 490 495

Glu Glu Pro Lys Tyr His Gly His Ala Leu Gln Leu Leu Arg Gly Asn
 500 505 510

Thr Thr Gly Thr Gln Ser Val Leu Trp Gly Ala Gln Ile Gln Asn Gly

515

520

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Thr Ala Ala Ser Val Leu Asn Leu Leu Ser Ile Arg Arg Arg Gly Thr
530 535 540

Phe Ser Leu Ser
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